

***P***ROCEEDINGS

*American  
Academy  
of Forensic  
Sciences*



*73<sup>rd</sup> Annual Scientific Meeting  
Held Virtually  
February 15-19-2021*



## AMERICAN ACADEMY OF FORENSIC SCIENCES

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### ***PROCEEDINGS***

of the American Academy of Forensic Sciences 73<sup>rd</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, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

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# ***PROCEEDINGS***

**of the American Academy of Forensic Sciences**

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## **TABLE OF CONTENTS**

Special Sessions .....	1
Case Break Sessions .....	4
Workshops .....	9
Scientific Sessions	
Anthropology .....	29
Criminalistics .....	146
Digital & Multimedia Sciences .....	310
Engineering & Applied Sciences .....	342
General .....	375
Jurisprudence .....	468
Odontology .....	500
Pathology/Biology .....	524
Psychiatry & Behavioral Science .....	674
Questioned Documents .....	704
Toxicology .....	739
Last Word Society .....	792
Young Forensic Scientists Forum Posters .....	796
Financial Disclosure Indexes .....	811
Key Word Index .....	823
Presenting Author Index .....	835

## S01 To See or Not to See: Unbiased Answers to Forensic Questions

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the role of human bias in forensic sciences, the risks and benefits of information, targeted versus non-targeted methods, decoupling data analysis and comparison, the standardization of methods, and a greater appreciation for the complexity of balancing contextual information and decision points in forensic science disciplines and its impact in the justice court system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering the various perspectives regarding the role of human bias in forensic sciences and the pursuit of truth and justice.

Forensic science can be a powerful force for truth and justice, but a dangerous instrument to subvert truth and justice when performed with bias. Forensic scientists strive to be neutral and objective and take steps to address bias. From television dramatizations, the public may have the wrong impression of how we do our jobs. Nonetheless, it is important that the public trusts the forensic science community. We need to be seen as neutral and objective, and, in fact, be neutral and objective. Forensic science disciplines vary in their susceptibility to bias. The most accusatory of the forensic sciences, fingerprint and DNA evidence, involve objectively searching electronic databases without a specific suspicion of a given individual. With further automation, the forensic science community will become further freed from bias and an ever-greater force for truth and justice.

This workshop will explore the human bias in targeted and non-targeted approaches in forensic science. This workshop will discuss decoupling data analysis and comparisons and the measuring of forensic identification information, with a special look at a DNA case example to evaluate the impact of targeted versus non-targeted methods on justice.

Medical examiners have statutory duties to determine cause and manner of death and must obtain multiple sources of information that may be hearsay in a criminal trial. This process can limit targeted investigations, as the medical examiner has a statutory duty to investigate some deaths and not others. An autopsy and its findings cannot be viewed in isolation of other information to get the correct cause and manner of death. Forensic pathologists may have information withheld that biases or limits their opinion. This can result in disagreements between the forensic pathologist, who typically will present the evidence in a court of law, and those who are certifying cause and manner of death.

There are multiple sources of bias, such as analytical bias, test selection bias, result interpretation bias, and case interpretation bias. Analytical bias can be easily managed by employing validated methods and reporting uncertainty of measurement where appropriate. However, there will be instances in which the expert has to exercise their discretion based on their expertise. Those decisions should be made objectively based on data. Contextual information guides test selection, which may be prone to bias. Toxicological data, for example, cannot be interpreted in a vacuum, case information is always required to evaluate the role of substances in a case. Since contextual information is required to appropriately interpret toxicology findings, it is essential that the scientific basis for opinions and conclusions always be provided.

By Rules of Evidence and the interpretive case law dealing with scientific and technical expertise, the judicial system controls whether expert opinion can be admitted as evidence in cases and how such evidence is judged to be adequate for purpose. Despite the *Daubert* decision in 1993 that purported to judge scientific evidence by the scientific method, older methods of attributing validity to expertise, such as legal precedent and “common sense,” often create inconsistent and unpredictable results.<sup>1</sup> These are forms of bias in need of correction. *Daubert* specifically drew attention to the need for applied standards in scientific evidence. The judicial system has regarded confrontation, cross-examination, and discovery as the “crucibles of truth” by which scientific truth can be discerned. Increased attention is being given to the development of standards-based assessments of validity, reliability, and the effects of biases of many sorts on the quality of evidence that should be trusted in cases at law. As the judicial system refines its standards for determining the proper way of determining adequate justification for assertions of truth based on science or other knowledge-based expertise, forensic science also seeks to refine its warrant of believability through standardized methodologies based on validated and demonstrably reliable standards of practice and conduct.

The importance of the implementation of standardized methods of analysis in pursuit of justice and truth in evidence will be discussed. This presentation will cover the steps necessary for the development and implementation of standards, including the incorporation of inter-laboratory exercises in the development of documentary standards. In addition, a summary of new developments and the current state of standardization of practice within the forensic chemistry disciplines will be presented.

### Reference(s):

<sup>1</sup> *Daubert v. Merrell Dow Pharmaceuticals*. United States Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.

### Eliminating Bias, Non-Targeted methods, Standardization in Reducing Bias

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### **S02 Young Forensic Scientists Forum (YFSF) Special Session—One Academy Pursuing Justice Through Truth in Evidence ... the Truth, the Whole Truth, and Nothing But the Truth**

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**Learning Overview:** After attending this presentation, attendees will better understand forensic science, how anyone's work can result in testimony, and how the different pressures that different courts impose can impact and shape our work, 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 YFSF has historically provided a venue for those forensic scientists with less than five years of professional experience to connect 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 future young scientists.

This year's special session provides valuable information to young forensic scientists regarding the various disciplines within the American Academy of Forensic Sciences (AAFS) and will serve as a forum to exchange, discuss, and cultivate new ideas. This full-day program has been put together to cover a wide range of topics and inform attendees as to how these topics relate to downstream disciplines, techniques, and individuals. The YFSF aims to show all young forensic scientists that what makes us special isn't just who we are alone, but how and what we contribute to forensics and the court system.

Every year, the AAFS draws in thousands of globally recognized experts and professionals to present research, hold workshops, and showcase the various disciplines represented within the Academy. This session will feature speakers from many of the AAFS sections who will discuss their work and how this work resulted in their presenting evidence in a court of law—both domestically and internationally.

Through these presentations, attendees will learn how experienced practitioners and professionals have worked to forward their disciplines and forensics as a whole and how their work culminated in presentation of evidence to a court during testimony. Through this special session, forensic experts will provide mentorship to young forensic scientists by conveying their experiences, their successes, their failures, and the lessons they learned.

The YFSF has historically provided a venue for those forensic scientists with less than five years of professional experience to connect 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 future young scientists.

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#### **YFSF, Special Session, Young Forensic Scientists Forum**



## SC01 The Implementation of Forensic Science Standards in an Operational Setting: Challenges and Solutions

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**Learning Overview:** After attending this presentation, attendees will understand the challenges and solutions associated with the implementation of forensic science standards in an operational setting and will gain increased awareness regarding specific resources that are available to facilitate their implementation efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing greater public awareness of the existence of forensic science standards and positively contribute to the advancement of the profession.

The 2009 National Academy of Sciences (NAS) Report highlighted the need for the forensic science community to embrace the adoption of consensus-based standards (“... standards and best practices create a professional environment that allows organizations and professions to create quality systems, policies, and procedures and maintain autonomy from vested interest groups. Standards ensure desirable characteristics of services and techniques such as quality, reliability, efficiency, and consistency among practitioners.”).<sup>1</sup> During the past decade, significant progress has been made in this respect. Existing Standards Developing Organizations (SDOs) and newly created SDOs have championed these efforts, in partnership with the Organization of Scientific Area Committees for Forensic Science (OSAC) of the National Institute of Standards and Technology (NIST). According to a 2020 survey of the AAFS membership, 89% of respondents were aware of standards development activities within their disciplines. Support for these activities were >80% across all sections of the Academy and >90% across all levels of membership (Student Affiliates to Retired Fellows).

In this interdisciplinary symposium, operational laboratories share the challenges and solutions associated with standards implementation across a variety of scientific disciplines. Five recently published or updated standards from the Academy Standards Board (ASB) and American Society for Testing and Materials (ASTM) International will be used to demonstrate how operational laboratories overcame a variety of implementation challenges. Using illustrative examples in toxicology, DNA, seized drug analysis, disaster victim identification, and interdisciplinary training standards, leaders in operational laboratories will share their practical experience with standards implementation from within their organizations.

Finally, resources for laboratories who adopt (or who have already adopted) those standards will be shared using the OSAC Implementation “Toolkit.” This process allows individual forensic science service providers that formally adopt consensus-based standards to be formally acknowledged, but it also enhances greater public awareness of the existence of the standards themselves and positively contributes to the advancement of the profession.

**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, Implementation, Forensic Science



### CB01 Forensics Standards Development: What's New With Standards Developing Organizations (SDOs) and What is the Organization of Scientific Area Committees (OSAC) for Forensic Science's Role

Karen Reczek, MLS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Teresa Ambrosious, BS\*, AAFS Academy Standards Board, Colorado Springs, CO 80904

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**Learning Overview:** After attending this presentation, attendees will have learned about the standards development process in the United States and internationally, as well as the advances in forensic standardization from United States and international SDOs, including the American Academy of Forensic Sciences (AAFS) Standards Board (ASB), the American Society for Testing and Materials (ASTM) International, the American Dental Association (ADA), the National Fire Protection Association (NFPA), the international SDO, the International Organization for Standardization (ISO), the International Organization for Standardization, and other relevant SDOs. Attendees will gain a better understanding of the progress of consensus standards development in different forensic disciplines, including fire debris, seized drugs, biology, digital evidence, toxicology, friction ridge, blood stain pattern, footwear/tire tread, crime scene investigation, and fire investigation. Attendees will learn how the Organization of Scientific Area Committees (OSAC) for Forensic Science working to facilitate the development and encourage forensic science service providers to implement them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating forensic professionals on new and emerging standards in the various forensic disciplines as they can be implemented in the laboratory setting, incorporated into quality manuals, be applied to accreditation scopes, and can assist in further validation of methods and techniques in inter-laboratory studies. The 2009 National Research Council (NRC) stressed the lack of standardization in forensics.<sup>1</sup> The advent of the OSAC administered by the National Institute of Standards and Technology (NIST), and the ASB has resulted in several years of concerted efforts to identify gaps in standards and begin their development using the voluntary consensus standards development process.

In 2009, the NRC published *Strengthening Forensic Science in the United States: A Path Forward*, which summarized the challenges facing the forensics community and the lack of mandatory standardization, certification, and accreditation.<sup>1</sup> From this report, the Department of Justice (DOJ) and NIST signed a Memorandum of Understanding, which led to the establishment of The National Commission on Forensic Science (NCFS) and the NIST-administered OSAC for Forensic Science.

This presentation will provide an overview of the standards development processes in the United States and internationally. Informative content will include recent progress in standards development in forensic sciences. Attendees will learn about standards currently in development, soon to be published, and new standards from various SDOs, such as the ASB, the ASTM International, the ISO, the ADA, the NFPA, and other relevant SDOs. New standards include interdisciplinary topics, such as evidence collection and labeling, interpretation and reporting, and discipline-specific standards in the areas of criminalistics, digital evidence, facial recognition, photography, biology, pattern evidence, and crime and fire scene investigation with a focus on terminology, measurement uncertainty, measurement traceability, quality assurance, and calibration.

The OSAC for Forensic Science has been working closely with various SDOs to bring the community together to define requirements for the various disciplines for submission to the standards development process. The various roles in the standards development process and promotion of implementation of these standards will also be covered.

#### 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, OSAC, Implementation



### **CB02 The Sarah Yarborough Homicide—The First Case Where Investigative Genetic Genealogy Was Used to Generate Forensic Intelligence in 2011**

*Colleen M. Fitzpatrick, PhD\*, Fountain Valley, CA 92708*

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the development of investigative genetic genealogy as a means of developing investigative intelligence for cold casework. The 1991 homicide and sexual assault of Sarah Yarborough was the first known instance where direct-to-consumer DNA test data was used to generate investigative leads for a cold case, opening the door for countless others that have since been solved using genetic genealogy. The Yarborough homicide was first attempted in 2011 by comparing the Y-chromosomal Short Tandem Repeat (Y-STR) profile obtained from the crime scene DNA to public Y-STR genetic genealogy databases. When the case was solved in 2019 using autosomal Single Nucleotide Polymorphism (SNP) testing GEDmatch, it was discovered that Sarah's killer could have been identified at least 20 years earlier through the Combined DNA Index System (CODIS), but loopholes in the legal system had allowed him to avoid detection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proving a case study that illustrates the strengths and weaknesses of forensic genetic genealogy compared to those of conventional STR typing, conventional genealogical research, and the legal system, and how each factored into the resolution of 30-year-old cold case in spite of the loopholes and flaws in the system.

In 2011, a match was found for the killer's Y-STR profile to members of the Fuller Y-STR surname project who were descendants of Robert Fuller of Salem, MA, in the 1630s, a relative of the Mayflower Fullers. Suspicion fell on William Fuller, a long-time Yarborough family friend, who had been in the area at the time of the murder and whose daughter Elizabeth was Yarborough's classmate. When William Fuller voluntarily gave a DNA sample, it was determined that he was not the killer nor was he the father of the killer. However, his Y-STR profile matched the Y-profile from crime scene DNA, indicating he was a paternal cousin of the killer, although it was not possible to estimate how closely they were related. The unusual situation developed that although the killer was still unknown; authorities knew his genealogy back to the 1600s and had even identified a cousin. Fullers living in the area were investigated but the case went cold again.

The 2019 identification of Patrick Nicholas as a suspect using genetic genealogy autosomal SNP testing raised awareness of the limitations of CODIS and fueled debate over the role of familial searching versus genetic genealogy. Nicholas was convicted in 1983 of attempted first-degree rape in Benton County, WA, before CODIS was launched in the 1990s. In 1993, he was arrested again for first-degree child molestation. Although his DNA profile should have been entered into CODIS, he was allowed to plead to gross misdemeanor that did not require DNA collection. He escaped detection a second time. After Nicholas' arrest, it was discovered that his brother Edward had already been entered into CODIS for a prior conviction for rape in the first degree; he was also a registered sex offender. Because Washington does not practice familial searching, Patrick Nicholas had escaped detection a third time.

Upon Nicholas's identification using genetic genealogy, the King County Sheriff's Office quickly secured his DNA from discarded cigarettes. His DNA was found to be a CODIS match to the DNA profile developed from the victim. Nicholas has been charged with first-degree murder with sexual motivation. He is currently pending trial in King County Superior Court, Seattle, WA.

Ironically, Sarah's killer was named Nicholas, not Fuller. His grandfather was adopted, so his legal surname was not his biological surname, highlighting the fact that even genetic genealogy has its loopholes.

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#### **Genealogy, CODIS, SNPs**



## CB03 Bail Reform or Bail Elimination: A Historical Perspective and Case Analysis

*Patricia C. Smith, MSL\*, Harris County District Attorney's Office, Houston, TX 77002; Betsy Adelizzi, PhD\*, Fulshear, TX 77441*

**Learning Overview:** The goal of this presentation is to create an open and honest dialogue about the criminal justice system and those taxed with the responsibility of ensuring a fair and just system through inclusion, diversity, and public safety in mind.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reminding all attendees of their role in making positive and effective changes to the criminal justice system by focusing on inclusion, public safety, and fairness to all parties through collaborative research and practice with multiple disciplines.

The bail system has been around for hundreds of years. Criminal justice was a “largely private, often brutal affair. Family members were expected to avenge their murdered kin. Any private citizen could kill an offender sentenced to “outlawry.”<sup>1</sup> Anyone caught in the act of committing a crime could be summarily executed.”<sup>1</sup> Gradually, personal blood feuds were exchanged for financial compensation to the victims by the offenders. There was a transition to a court-controlled justice system where ensuring appearances became a priority.

The offenders were tasked with finding another person to serve as a surety for the accused crime. However, the Industrial Revolution brought about more movement. Sureties were often family members or friends of the accused. Increased travel made locating sureties more challenging and created an incentive to flee the jurisdiction during trial or prior to verdict. In 1898, England passed the Bail Act to replace the previously required personal surety.<sup>2</sup> England proposed the Bail Act would be a more effective method of ensuring court appearances and curtail recidivism.

The United States did not initially enact bail legislation; instead, it produced the first commercial bondsmen. Bail bonds became a lucrative business and quickly sparked interest across the country. Many courts were requiring bail to be paid in full as a condition of release.<sup>2</sup> Offenders reached out to bondsmen who paid the bail in exchange for interest and full repayment. The courts continued to increase the use of cash bail; more people became reliant on bondsmen. This quickly became about money and less about ensuring appearances at future court dates.

The American Criminal Justice System, under the guidance of the Eighth Amendment Bail Clause, continue to evolve its bail system by adding different types of bonds.<sup>3</sup> The most common bonds are Surety Bonds, Personal Bonds, and Cash Bonds. Additionally, there are Cash percentage of Bond and General Order Bond. Most states utilize at least one of the previously mentioned bonds.

A Surety Bond is a promise by a bail bond company/agent to pay one party a certain amount if the second party fails to meet the obligation(s); the surety bond protects the obligee against losses resulting from the defendant's failure to appear and typically requires a non-refundable fee (usually 10%).<sup>4</sup>

A Personal Bond (Own Recognizance) is no money down and the person must agree to appear at future court dates. Personal Bonds are typically used for minor offenses and offenses where there is little to no threat to the public. A General Order Bond is treated the same.

A Cash Bond is paid in its entirety. A Cash Bond is where payment of the entire amount of the bond is given to the court clerk. The money can be returned at the end of the case if the defendant does not violate his/her bail agreement.

A Cash percentage of Bond is like a Cash Bond, but only 10% of the bond is given to the court clerk. Money will be returned after disposition/end of case/trial unless the person violates their bond conditions. Should the defendant violate the bail agreement, the State can seize property that will cover the entire amount.<sup>5</sup>

The individual states have evolved to the point of modifying, if not arguably eliminating, their state's bail system based on politics and capitalism. This presentation will review California's, Texas', South Carolina's, and New York's previous bail system, including comparing their current changes. This presentation will discuss specific Harris County cases and engage the audience through polling.

### Reference(s):

1. Seibler, John-Michael and Jason Snead. The History of Cash Bail. *The Heritage Foundation*, August 25, 2017. <https://heritage.org>.
2. Jackson, Angel. The Evolution of Money Bail Throughout History. *W. Haywood Burns Institute*, April 18, 2016. <https://burnsinstitute.org>.
3. U.S. Const. art. VIII.
4. McCullough, Jolie. Harris County Agreed to Reform Bail Practices that Keep Poor People in Jail. Will it Influence Other Texas Counties? *Texas Tribune*, July 31, 2019. <https://www.texastribune.org>.
5. Morrell, Jane. 4 Types of Bail Bonds in Rock Hill, South Carolina. Accessed May 28, 2020. <https://www.mlblaw.com/blog/types-bail-bonds/>.

### Bail, Bail Reform, Bail Elimination



## CB04 Lessons Learned: The Exoneration of Robert Earl DuBoise

*Susan Friedman, JD\*, Innocence Project, New York, NY ; Teresa Hall, JD\*, Hillsborough County State Attorney's Office, Tampa, FL; Adam J. Freeman, DDS\*, Westport, CT 06880; Nancy Dinh, MS\*, Forensic Analytical Crime Lab, Hayward, CA 94545*

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**Learning Overview:** The goals of this presentation are to help attendees: (1) understand the factors that lead to wrongful conviction; and (2) understand how through a collaborative process the prosecution, defense, and forensic experts can search for the truth.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the numerous factors that led to the wrongful conviction and death sentence of a young man for a rape and murder he did not commit. Attendees will hear about how bitemark evidence triggered Robert's wrongful arrest and the impact of that conclusion on the rest of law enforcement's investigation, the role of the prosecution, defense, and forensic experts in the search for the truth, and what has happened in the aftermath of the exoneration.

In the early morning hours of August 19, 1983, a young woman's body was found behind a dentist's office in Tampa, FL. The victim was covered in blood and had a severely beaten face and jaw. The scene also indicated that the woman had been a victim of an actual or attempted sexual assault: she was lying on her back, nude except for a tube top that had been pulled over her chest, exposing her breasts.

The Hillsborough County Medical Examiner (ME) conducted an autopsy, and the cause of death was determined to be massive blunt force trauma to the head. During the autopsy, the ME noticed what he believed to be a bitemark on her left cheek. A dentist on contract with the ME's office also examined the injury on the victim's face and concluded it was a human bitemark. Lastly, the ME collected a rape kit.

Detectives interviewed a number of witnesses, but none offered a solid lead. With no eyewitnesses to the crime, police quickly focused on obtaining identifications from individuals. Robert was among those who provided an impression. A board-certified forensic odontologist examined the alleged bitemark and the impressions and concluded that Robert's teeth caused the mark. No other physical evidence connected Robert to the crime.

With its entire case resting on bitemark evidence as the case headed to trial, the State utilized a jailhouse informant to build its case. Ultimately, Robert was convicted of murder and attempted sexual assault. The jury recommended a life sentence but the judge overrode the jury's recommendation and sentenced Robert to death. The Florida Supreme Court subsequently vacated the death sentence.

Robert filed a motion for post-conviction DNA testing in 2006, but after a hearing, he was notified that the vast majority of the evidence in his case was destroyed in 1990—just five years after his conviction. In 2018, the Innocence Project began investigating Robert's case. Among the concerns was the use of bitemark evidence. In 2019, the Innocence Project contacted the Conviction Review Unit at the State Attorney's Office. Together they engaged in a collaborative process in a search for the truth. As part of this process, they had the bitemark evidence and testimony reexamined, and an independent examination by a forensic odontologist concluded that the mark on the victim's face lacked sufficient detail and that there is no scientific support that odontologists could reliably diagnose a bitemark. Moreover, the methodology used in the evidence collection, analysis, and comparison demonstrated bias and was not in keeping within techniques of the time. Additionally, despite claims that the rape kit was destroyed, slides created during the victim's autopsy were found—critically, semen was identified on the victim's vaginal smear slide, Robert was excluded, and there was a presumptive hit in the Combined DNA Index System (CODIS).

On September 14, 2020, Robert's conviction was vacated. The judge found that the newly discovered DNA evidence and the newly discovered evidence about the bitemark evidence each entitled Robert to relief and concluded that Robert was innocent.

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### Exoneration, Bitemarks, Duty to Correct



## CB05 The ABCs of TLC

*Irina Geiman, MS\*, United States Secret Service, Washington, DC 20223; Julia M. Barker, MSFS\*, United States Secret Service, Washington, DC 20223*

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**Learning Overview:** After attending this presentation, attendees will understand the basic scientific methodology for the forensic analysis of inks using Thin-Layer Chromatography (TLC). Attendees will have an opportunity to observe and learn analytical steps taken prior to and during the chemical examination of writing inks and printing inks typically found on questioned documents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of robust ink analysis and the limitations and interferences commonly encountered in such analysis. This presentation will provide a brief overview of microscopic and alternate-light source examinations, but will focus on TLC techniques utilized in the comparison and discrimination of inks. This presentation is intended as an introduction to ink analysis and will not address many of the scenarios and limitations commonly encountered in forensic casework.

Analysis of writing inks and printing inks typically involves comparison of inks to a potential source, such as an ink cartridge, to each other, or to a collection of standards. It is a multi-step process, which begins with a non-destructive physical examination before progressing to destructive chemical analyses necessitating sample removal. While the examination may be halted at any step of the process, it is important to consider the level of discrimination that may be achieved if additional analytical methods were to be employed. For example, TLC often does not differentiate pigmented inks; therefore, a technique such as Raman spectroscopy may be considered.

Ink examination also requires a well-developed understanding of limitations of the employed analytical techniques. Even basic light microscopy used to determine writing instrument type may provide inconclusive results when examining “hybrid” inks. The complexity of interfering factors increases with each subsequent analytical step. For example, during TLC analysis, Ultraviolet (UV) components of the substrate may interfere with UV components of the ink, creating overlapping bands on the TLC plate.

Attendees will also learn about several well-established methods available for the dating of inks. While ink dating may provide valuable information, it presents a host of issues, such as availability of reference materials and storage conditions that may affect document aging. This presentation will address some of the overarching concerns that should be evaluated when considering ink dating analysis.

During this presentation, attendees will have an opportunity to observe the non-destructive physical and optical examinations conducted prior to the destructive TLC analysis. Attendees will observe commonly encountered interferences such as sample size limitations, substrate fluorescence and staining, and TLC plate effects. This presentation is intended to provide baseline knowledge of the scientific methodology used in the forensic examination of inks and will assist examiners in determining when chemical analyses would be beneficial for sample discrimination.

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### **Ink Analysis, TLC, Thin-Layer Chromatography**

### **W01 A Complete Introduction to Digitally Captured Signatures (DCS) and a Tutorial for Namirial's Firma Certa Forensic Analysis Tool**

*Niko Kalantzis, MSc\*, Charotularios PC, Piraeus, Attiki 18535, GREECE; Samiah Ibrahim, BSc, Ottawa, ON K1S 1R1, CANADA; Timothy Campbell, BSc, Canada Border Services Agency, Ottawa, ON K2E7M6, CANADA*

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**Learning Overview:** The goals of this workshop are to introduce attendees to the new era of DCS, present the legal aspects of the new “digital” documents in reference to traditional pen and paper products, and familiarize attendees with the intricacies of handling such products, both from the hardware and the software aspect. Attendees will acquire the basic knowledge of DCS data and its correspondence to traditional pen and paper signatures and will gain experience using a DCS forensic analysis tool.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees understand and familiarize themselves with DCS as a medium. Attendees will know what to look for in a case, how to analyze the DCS, and how to use this highly advanced forensic tool.

DCS are a new technology in the field of Forensic Handwriting Examination. DCS are increasingly used throughout the world, with many countries adopting electronic documents encrypted with DCS biometric data as legally binding documents equivalent to traditionally signed paper documents. Furthermore, for the first time, DCS provide the expert with quantitative insight to features and characteristics of signatures (such as pressure and velocity) that were previously only qualitatively approached. This new technology can help train the expert to more accurate evaluation of dynamic aspects of signature execution on paper through training on hybrid signatures (signatures executed with pen on paper but at the same time captured on a digitizer).

This workshop introduces the new DCS technology to the experts and familiarizes them with the basic aspects and principles of hardware and software issues as well as legal aspects of such biometric documents. The methodology of examining traditional pen and paper signatures is transposed into the new information provided by the DCS technology and introduced to the experts; practical examples will be introduced.

In order to familiarize themselves with the actual handling and analysis of DCS biometric digital documents, attendees will have access to Namirial's Firma Certa Forensic software for DCS and will be trained in the use of its basic features. Specifically, attendees will be taught how to encrypt and decrypt collected samples, visualize the collected data, and apply the different available analysis tools.

Attendees will need to download and install the Firma Certa Forensic software (links will be supplied) on their computer. Access to a compatible digitizer tablet is desirable but not necessary.

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#### **DCS, Biometric, Signatures**



## W02 Interpreting and Communicating DNA Evidence in a Probabilistic Genotyping Universe

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**Learning Overview:** After attending this presentation, attendees will better understand the Likelihood Ratio (LR) and the limits of probabilistic genotyping and will gain tools to help explain the results of such analyses to stakeholders. The concepts to be discussed are applicable to probabilistic genotyping software in general and fundamentals of genetics and statistics. In addition, concepts of adventitious hits, false positives, and false negatives will be addressed. The relationship between a numerical result and the reliability of DNA evidence will be discussed. The appropriateness and limitations of a Bayesian framework for DNA evidence will be considered.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through: (1) attendees gaining a better appreciation for the benefits and limitations of probabilistic genotyping; (2) attendees gaining an understanding of the role of proposition setting; and (3) attendees gaining experience on ways to communicate findings to other scientists, investigators, and the judicial system.

Forensic DNA typing has been called “the gold standard” of the forensic science disciplines.<sup>1</sup> However, there is a resurgence of focus on the limits of the interpretation and testimony related to the quality of the profile and the numerical weight that accompanies an inclusionary interpretation.<sup>2,3</sup> Indeed, there is some debate as to whether an interpretation of inclusion/exclusion is even appropriate.<sup>4</sup> Due to the implementation of probabilistic genotyping approaches, mutually exclusive hypotheses and expressing the significance of analysis in the form of the LR are being adopted in the United States.

Because of the need to better interpret mixture evidence and the availability of software tools, probabilistic genotyping has been embraced to assess a DNA mixture profile from the perspective of the genotypes from individual donors. When there are multiple donors, there can, and likely will, be many more potential genotypes to explain the mixed DNA profile than there are donors that contributed to the profile. While previously laboratories tended to attempt to deconvolve mixtures of two persons into single-contributor-possible genotypes, modern probabilistic genotyping software has the ability to deconvolute mixtures of three-, four-, or five-person mixtures. In theory, there may be no limits to the number of contributors.

There are numerous benefits to generating a probabilistic LR, such as the ability to: (1) interpret previously deemed inconclusive profiles that may support exculpatory results; (2) interpret mixtures that were considered too complex; (3) provide more consistency and less variance in mixture interpretation among analysts; (4) obtain more data to effect an interpretation; and (5) use the LR from a single locus even if  $<1.0$  while other loci support a contributor hypothesis.

The use of probabilistic genotyping software requires understanding of such data and how to express the data in a cogent fashion. For example, some samples that used to be considered as “inconclusive” may be evaluated, which may result in a relatively low LR and a discussion as to whether low LR is reliable. Low “numbers” have always been a part of the process, including with the Random Match Probability (RMP) and Combined Probability of Inclusion (CPI). Expression of the results can be challenging, especially when the experience of analysts and the judicial system is based on traditional methods of interpretation. There are commonalities and differences in the various methods of interpreting DNA profiles, and they need to be appreciated to support the use of advance tools, such as those for probabilistic genotyping, so that evidence can be conveyed in a meaningful and effective manner.

The overall and components of the LR and how the LR is used in a Bayesian framework shall be explained fundamentally and with examples.

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### Forensic DNA, Probabilistic Genotyping, Bayesian Framework

## W03 Scientific Working Group on Digital Evidence (SWGDE) and Digital Evidence: The Look of Modern Criminal Investigations

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**Learning Overview:** The goal of this presentation is to provide attendees with an overall view of the various types of digital forensic examinations and technical work being performed by members of SWGDE that can be applied in the attendees' day-to-day work.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness among the general population of forensic scientists of the work in digital forensics and by making digital forensic practitioners aware of the useful resources, guidance, and best practices available from SWGDE.

As technology continues to evolve and morph into new formats and platforms, the challenge of digital and multimedia forensics progresses as well. Digital forensics experiences dynamic changes caused by the rapid progression of technology, new inventions with new applications, and the continued miniaturization and complexity of the technologies arriving to digital forensics labs. While many forensic science disciplines encounter iterative change enabled by new methods and new technologies, digital forensic science encounters revolutionary change across devices and technologies not yet created.

The market forces driving adoption of new technologies heavily affect digital forensic practitioners. As emerging technologies arrive to market, these same new technologies begin to appear in evidence queues to be addressed by forensic practitioners. Similarly, as new technologies such as cloud providers, social media, data storage devices, and market data tracking capabilities become available, digital forensic techniques can be advanced as well in their implementation in existing forensic processes. New methods and research exist in emerging technology areas that can inform discreet problem statements while finding applicability across other new technology as well.

Digital forensic casework faces a continual evolution of technological challenges—challenges emerge daily with how to successfully perform validated forensic examinations on new platforms. The growth in technology and data storage techniques not only work against a digital forensic practitioner, but additionally provide for the development of new capabilities to further their forensic analyses. New forensic methods and best practices are constantly emerging as new types of data and devices that have not yet been previously encountered in forensics labs become commonplace.

The SWGDE is comprised of more than 80 members representing all levels of the digital forensic community who are dedicated practitioners working diligently to provide guidance and best practices to address relevant technological issues. These members have encountered examinations that evolved into unique solutions that will have a positive and important impact on current forensic examinations and will provide valuable solutions.

As new forensic techniques are being researched, developed, and documented as best practices, processes being used to validate these methods must also grow in development and implementation. Garfinkel identified that standardization and validation must be strengthened to meet the challenge coming with future technologies.<sup>1</sup> Lillis et al. identify that future digital challenges in and of themselves can hamper investigations, but the cumulative effect amplifies these difficulties.<sup>2</sup> Caviglione et al. call out the need for multidisciplinary approaches spanning multiple fields to address the future of digital forensics.<sup>3</sup>

After attending this workshop, attendees will be able to: (1) list examples of new emerging technologies and forensic methods used against them, (2) articulate challenges related to the digital forensics of new technologies, (3) discuss methods, processes, and strategies for addressing software testing and validation, and (4) have the opportunity to review existing literature, case studies, and research areas related to forensic and validation challenges.

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### Digital Forensics, Software Validation, Multimedia

## W04 The Impact of the COVID-19 Pandemic on Forensic Sciences With a Focus on Opioids and Emerging Drugs

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of: (1) the challenges that have resulted from the COVID-19 pandemic in the management of patients with Opioid Use Disorder (OUD); (2) the measures that institutions and providers have taken to address those challenges; (3) how toxicology and seized drug analysis have been impacted and how they have adjusted to meet unprecedented demands and challenges; and (4) how a COVID-19-impacted court system struggles to maintain the balance of justice and procedural law in the midst of interrupted and shut down of court services.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by offering a multidisciplinary perspective on the impact of the COVID-19 pandemic and its effects on opioid use and emerging drugs in the United States.

The COVID-19 pandemic has created new challenges in the management of patients with OUD.<sup>1</sup> Opioid overdoses and related deaths are increasing in various jurisdictions across the United States, likely due to increased psychological stress from isolation, employment- and income-related problems, and reduced access to mental health services, as well as the decreased administration of naloxone. To counteract these issues, the Substance Abuse and Mental Health Services Administration (SAMHSA) has advised take-home medication for drugs that previously required in-person administration. In addition, the Drug Enforcement Administration (DEA) will allow providers to evaluate patients more flexibly (for example, through telemedicine or phone interviews) and to prescribe Medication-Assisted Treatment (MAT) without written prescriptions.<sup>2</sup>

Law enforcement was also affected, by the re-allocation of resources to public safety activities, as a result of stay-at-home orders and civil unrest. Even as road traffic volumes went down, there was an early spike in fatal motor vehicle collisions as reckless driving increased, many incidents of which involved alcohol and drugs.<sup>3-5</sup> Year-over-year traffic fatalities nationwide were up 14% in April 2020 as compared to 2019.<sup>6-8</sup> Homicide rates also increased, possibly due to a spike in gun purchases in the early days of the pandemic, boredom, and social displacement.<sup>9,10</sup>

Toxicology laboratories initially saw a decline in Driving Under the Influence (DUI) and Driving Under the Influence of Drugs (DUID) casework, as enforcement focused on more pressing public safety issues. However, drinking and drug use and driving arrests soon returned to pre-pandemic levels, as restrictions eased. Postmortem toxicology caseloads remained steady throughout the pandemic, since COVID-related deaths did not necessarily require drug testing. Drug-related deaths, however, increased in the first three months of the pandemic. Requests to test for popular COVID therapies, real and imagined, such as hydroxychloroquine/chloroquine testing, bleach and disinfectant ingestions, and the continued appearance of novel psychoactive substances increased laboratory caseloads.

Drug analysis in forensic laboratories were also heavily impacted. Agency policy and local stay-at-home orders affected the ability of laboratories to maintain operations. For example, in Cook County, IL, in March 2020, prosecutors were instructed to dismiss all pending drug cases, if forensic laboratory testing had not been completed, due to the closure of the Cook County crime lab.<sup>11</sup> While several laboratories reported early reductions in drug submissions, in later months, submissions resumed, often before the laboratory had fully reopened. Managers now had to address and implement protocols for social distancing, sourcing personal protective equipment supplies, staffing shortages, and absences due to illness, mandatory quarantine, and childcare responsibilities.

Across the United States, courts also shut down to avoid the risk of virus exposure to jurors, defendants, and court personnel. While pretrial hearings progressed in virtual courtrooms, trials were not permitted due to the right of the defendants to personally confront witnesses, and courts have almost universally held the needs to be in person, when requested by the defense. Judicial systems at local, state, and federal levels are coming back into operation in the midst of the COVID-19 pandemic, causing pronouncement of differential rules, deadlines, and decision-making. Courts now faced limited capacity mandates and handling operations with social distancing requirements, resulting in difficult decision-making processes as to which litigants and participants are granted court resources. Delay or denial of personal contact, orderly processing, and hope in the judicial system can be deadly for a person dealing with opioid addiction. As courts return to full power, Treatment and Problem-Solving Courts should be prioritized for return to personalized, comprehensive services for those suffering from mental health disorders, opioid addiction, and substance abuse disorders in general.

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### COVID-19, Forensic Sciences, Opioids

### **W05 Forensic Genetic Genealogy (FGG): Practical Aspects of Implementation for Law Enforcement and Criminalists**

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**Learning Overview:** After attending this workshop, attendees will have foundational understanding and experiential knowledge of FGG, also known as Investigative Genetic Genealogy (IGG). Unlike other workshops on the subject, this workshop will provide insight into end-to-end practical decision-making that law enforcement and criminalists face from before a sample enters the FGG pipeline through the final genealogical identification process. Topics covered will include: (1) the organization of an FGG unit, including the development of Memorandums of Understanding (MOUs) and criteria for case selection; (2) Single Nucleotide Polymorphism (SNP) data generation technologies and how the choice of which technology to use is influenced by the quality and quantity of the DNA sample available; (3) the impact that quality and quantity have on workflow decision-making and data-driven checkpoints; (4) a live training session using genealogy tools with sample data; and (5) a template for studies that are critical to the development of standard operating procedures and interpretation guidelines. As part of the training session, this workshop will provide insight into how to evaluate data and interpret results and will familiarize attendees with the features, capabilities, and limitations associated with databases such as GEDmatch, as well as look at what new or advanced tools are on the horizon.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing experienced insight and guidance on the implementation of FGG for criminal investigations. Practical recommendations will be presented on the construction of an end-to-end FGG workflow, along with suggestions on how it may eventually be incorporated into a well-established Combined DNA Index System (CODIS) pipeline.

This workshop draws together a multi-disciplinary panel of law enforcement personnel, genealogists, and criminalistics experts who deconstruct the implementation of an FGG (a.k.a. IGG) pipeline and provides attendees with a blueprint for how they can operationalize an end-to-end FGG workflow to generate investigative leads and conduct confirmatory testing. Although FGG has provided closure for more than 200 cold and contemporary cases, law enforcement agencies, the legal community, and criminalistics labs still have questions around the incorporation of kinship Single Nucleotide Polymorphism (SNP) analyses and genetic genealogy databases within an infrastructure of well-established forensic Short Tandem Repeat (STR) analyses and databases such as the Combined DNA Index System (CODIS). Forensic typing methods such as Capillary Electrophoresis (CE) and Next Generation Sequencing (NGS) are well defined; whether a sample is eligible for CODIS upload is made based on protocol. With the advent of FGG, however, the decision-making process is more complex. The technology required to generate a genetic genealogy SNP profile, whether by whole genome sequencing, microarray, or targeted NGS, is currently in the hands of private labs, not-for-profit labs, and genetic genealogy companies. Decisions on whether a sample could or should be used for FGG analysis are based on emerging sample quantity and quality guidelines, such as those stated in the Department of Justice (DOJ) Interim Policy on Forensic Genetic Genealogy, that are not always understood by law enforcement and legal authorities, even with input from those labs and companies. The goal of this workshop is to provide law enforcement and criminalists alike a deeper insight and practical know-how that will enable them to exploit this game-changing technology to enable the most responsible and effective generation of investigative intelligence.

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#### **Genealogy, GEDmatch, SNPs**

### W06 Tuning in to the Barking Dog: Actionable Surveillance in an Evolving Chemical Threat Landscape

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**Learning Overview:** After attending this workshop, attendees will better understand how current challenges for toxicological screening in death investigation cases extend to combating the proliferation and use of unregulated chemicals as weapons and where practicing toxicologists can help contribute to an effective national surveillance network through proactive recognition of emerging chemical threats.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by: (1) providing technical competence on threat agnostic approaches to recognizing and preventing or mitigating evolving chemical threats; and (2) putting this new competence into action through facilitated discussions and exercises to establish a framework for proactive information sharing and surveillance.

Since the emergence in the United States of New Psychoactive Substances (NPS) in late 2007, the United States and international forensic toxicology communities have grown increasingly savvy at recognizing this continually evolving threat. Unfortunately, as toxicological screening capabilities have advanced, so have those of enterprising chemists and businessmen and women in evading attempts at NPS regulation. Further, the widespread availability of both the starting materials and instructional materials have expanded the public health threat to an issue of prominent global security concern. Against this backdrop, steady progress toward elimination of declared stockpiles of chemical weapons in support of the Chemical Weapons Convention (CWC), a multilateral disarmament and non-proliferation treaty that entered into force in 1997, has also driven the deployment of capabilities to detect and respond to a broad range of weaponized chemicals. Despite the successes and continued progress toward chemical demilitarization, the norms against weaponizing chemicals enshrined within this treaty are challenged through continued uses of chemicals as weapons by both terrorists and states in ways that had not been envisioned during the negotiations that led to the CWC. This includes weaponization of broader types and classes of chemicals, some of which may be sourced from readily available sources, which are being combined with low-tech approaches to both making and dispersing chemical threat agents. Proliferators have diverse options to deploy chemicals as weapons, and the anonymity and the speed with which chemical structures that fall outside established regulatory control listings and control measures can be produced.

This new reality has exposed an urgent need for domestic and international decision makers to have access to technical tools and approaches that fundamentally change how they respond to and prosecute cases of alleged attacks using weaponized chemicals to effectively counter the ever-changing landscape of chemical threats. Success requires enhanced capabilities and threat agnostic approaches to generate answers at the speed of relevance. Innovative and enabling approaches are being realized by integrating capabilities in analytical toxicology, ultra-trace chemical characterization, advanced data analytics, and expertise at the technology/policy interface, but to be useful to decision makers, we also need to synthesize the information gleaned from this work to establish a functional surveillance network.

In this engaging and interactive workshop, the speakers will provide a comprehensive overview of the problem set and invite attendees to help establish nationwide surveillance to help United States decision makers actionably recognize, mitigate, and/or potentially source evolving chemical threats stemming from the global NPS trade. Speakers will discuss the chemical threat landscape and its evolution since the entry-into-force of the CWC, the many challenges faced in responding to and investigating recent domestic and international chemical incidents, and the importance of threat agnostic solutions to success. This workshop will highlight the central role of forensic investigation in combatting chemical threats and illustrate how, in combination with data collection, informatics, and enabling technology and policy, it may provide a basis for an actionable surveillance network for United States and international decision makers.

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#### NPS Surveillance, Threat Agnostic, Untargeted Analysis

### **W07 Applications, Implementation, and the Future of Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) in Forensic Laboratories**

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**Learning Overview:** The goals of this presentation are to inform attendees of: (1) the fundamentals of how DART®-MS operates; (2) the current applications of DART®-MS in forensic laboratories; (3) what the practical considerations for implementation of DART®-MS are, including site planning, validation, testifying, and designing a workflow; (4) resources available to assist in the implementation process; and (5) the current state of research in the field of DART®-MS.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing practitioners, laboratory managers, and legal personnel with the necessary information to understand the strengths, weaknesses, and capabilities of ambient mass spectrometry systems, specifically DART®-MS. This information is crucial for the laboratories that currently use the technology and for laboratories that are considering implementation of this technology.

The aim of this workshop is to provide the necessary information to understand or expand current knowledge of DART®-MS while providing perspectives that will allow attendees to develop strategies for implementation, validation, and the ability to confidently testify. DART®-MS is one of many existing Ambient Ionization Mass Spectrometry (AIMS) technologies and is one of the few that have been successfully applied to forensic science. The ability of the technique to analyze a wide array of samples in a matter of seconds with little-to-no sample preparation makes DART®-MS ideally suited for forensic disciplines such as drug chemistry, toxicology, and trace evidence.

In the forensic science community, DART®-MS has been utilized for over a decade with much of the use coming from the screening of samples for the presence of drugs. DART®-MS has been shown to be a powerful tool for the analysis of a range of drug classes and sample forms (i.e., powders, pills, etc.) that include illicit and pharmaceutical-grade materials. Other areas where DART®-MS has been deployed include inks, explosives, lotions, lubricants, paints, and general unknowns. The technology has also been applied to more unique forensic applications, such as speciation of woods and analysis of cosmetics. This workshop will provide the practitioner viewpoint on many of these applications. There is also a large, and diverse, body of ongoing research that is related to existing and new forensic applications. These efforts are focused on topics including instrument modifications for more repeatable analyses, understanding ion chemistries, coupling with miniature mass spectrometers for screening purposes, developing new data treatment methods allowing for chemometrics, and even 2D chemical imaging. These and other efforts will be discussed.

With the implementation of any new technology, such as DART®-MS, practitioners have to be familiar with the fundamentals. They must also be able to validate the instrumentation and be prepared to defend their analyses in court. This workshop will aim to address these needs by providing attendees with considerations for implementation as well as validation and defending the technology during testimony. The goal of this workshop is to provide current, new, and potential users of DART®-MS, or any AIMS technique, with important information from implementation through the future of the technology.

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#### **DART®-MS, Mass Spectrometry, Implementation**

## W08 Utilizing Best Practice in Forensic Education and Remote Learning

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**Learning Overview:** After attending this workshop, attendees will be able to utilize established methods and techniques recognized as best practice to enhance their approach to forensic science education. Attendees will actively engage with digital tools and practice classroom exercises designed for in-person, hybrid, and remote learning. Drawing on a range of international perspectives, all aspects of the workshop are intended to translate directly into the forensic classroom, regardless of whether that classroom is in-person or virtual.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing tools that will improve the educational content and experience of future forensic scientists. In addition, these tools can be adapted for continuing education, training, and professional development of current forensic practitioners. It is hoped that improved pedagogical approaches will strengthen the academic backbone of the forensic community and support greater cross-institutional collaboration.

In March 2020, the educational landscape changed almost overnight. Schools, colleges, and universities were forced to move all instruction into a remote, online environment. For those with minimal online teaching, the sudden shift was extremely disruptive. Educators adapted as best they could. In some instances, remote content delivery mainly mirrored traditional content delivery. Due to a range of pressures, many instructors recorded their usual lectures or delivered them via Zoom or other conferencing platforms unaltered from their original format rather than adapting instructional content to best suit online learning. The result was often frustration for both instructors and students as well as questions as to whether learning objectives were met, particularly in classes requiring in-lab or hands-on activities that were no longer feasible.

This workshop provides an opportunity for forensic science educators to reflect on and revisit evidence-based pedagogical practices with the aim of improving instruction in the new modes of instruction now commonplace across institutions. In addition, new approaches to instruction and assessment in an online environment will be presented as well as specific applications for forensic science content delivery. Throughout the workshop, these methods and activities will be modeled by the presenters, and attendees will have the opportunity to test out the various tools and techniques presented.

The workshop will cover three main themes: (1) Effective Teaching Practices; (2) Best Practice for Remote Teaching; and (3) Digital Teaching Tools. Theme 1 will take attendees back to basic course preparation, including how to craft measurable learning objectives and how to align assessments to learning goals. Also addressed will be transparency in grading, including the creation of rubrics and how to facilitate active learning online. Finally, this theme will explore the spectrum of academic freedom and professionalization of teaching within higher education.

Theme 2 will dive deeper into the best methods and models of remote instruction. Various tools that enable accountability for reading and other asynchronous material consumption will be presented. This workshop will also cover how instructors can rethink assignments in an environment where laboratory access is restricted or prohibited. Finally, under this theme, ethical considerations when utilizing conferencing platforms and/or proctoring software will be discussed.

The final theme will present to attendees digital tools and how they can be incorporated into forensic instruction. Some tools such as the suite of Google® apps can be leveraged in new ways. Other tools will be new to attendees and have specific applications ideal for forensic content.

The move to remote instruction was dramatic; this workshop is designed to make the move less traumatic for forensic educators and the students they serve. By leveraging recognized best practice and the available digital tools, remote teaching cannot only be successful but can exceed the outcomes of traditional, lecture-based content delivery.

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### Education, Pedagogy, Remote Instruction



## W09 What They Don't Know Can Kill You

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**Learning Overview:** The goal of this workshop is to consider the function of standards in forensic disciplines and the ultimate role of forensic standards in the legal system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling attendees to understand: (1) how the legal community views and assesses the role of standards in court procedures; and (2) how standardization of methodologies and practices across an increasing number of forensic disciplines informs decisions by law enforcement and the courts. This presentation will alert forensic scientists on the need to engage in standardization activities to improve acceptability and use of forensic analyses, and, finally, this presentation will also raise the awareness of forensic laboratories to the advantages of voluntarily including newly developed forensic science standards in their procedures, even to the point of including them as part of lab accreditation to the International Organization for Standardization (ISO) standard #17025: General Requirements for the Competence of Testing and Calibration Laboratories.

The 2009 National Academy of Science (NAS) Report and the 2013 President's Council of Advisors on Science and Technology (PCAST) Report cast a shadow on the status of forensic science in the United States and identified the need for standardization to improve that status.<sup>1,2</sup> Even then, the forensic sciences involving DNA and toxicology were generally accepted sources of science-based information by law enforcement and the legal system—and they still are, but other forensic areas are eschewed as being less accurate and are, therefore, often considered unreliable. Lack of confidence is problematic when the acceptance and accuracy of evidence depends on information that is not available via DNA identification/exclusion or toxicology results. Too often, the scientific “truth” of evidence is left for the judge to determine, and attorneys are free to provide their own “scientific” interpretation. Indeed, under *Daubert*, the judge has become the gatekeeper for scientific evidence. Forensic scientists, on the other hand, conduct examinations, make identifications, record observations, perform analyses, and present results based on often rigorous procedures. Where, then, is the disconnect between the scientists and the legal system? What can standardization do to narrow the gap and increase court reliance on forensic results in a wider array of forensic disciplines as well as raise public confidence in both the legal and scientific communities.

Speakers from the Jurisprudence section will discuss the pros and cons of relying on forensic evidence and share their experiences, or lack thereof, and views with regard to forensic science standards. Forensic practitioners will discuss how they conduct their work, the role standards have in forensic determinations, and the difficulties of cross-jurisdiction acceptance of data. The head of a major crime laboratory organization will present results of an important survey detailing the use of forensic standards. Attendees will participate in lively question-and-answer sessions with the panelists aimed at drawing out additional information and identifying benefits and possible obstacles to increased use of forensic science standards.

### 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.

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### Standards, Forensic Science, Jurisprudence

### W10 When “Who” Doesn’t Matter as Much as “How”—DNA Testimony Given Activity Level Propositions

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**Learning Overview:** After attending this workshop, attendees will understand the need to separate the ability to help answer the “who” question from the “how” or “when” questions. Attendees will realize that in relation to DNA findings, it is important to recognize that the value (i.e., likelihood ratio) of DNA profiles when considering the source of the DNA will generally be completely different from the value of these results when considering the activities that are alleged to have happened.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guidance toward real-world testimony on how to handle DNA testimony when the court’s interest is beyond that of an “inclusion” and its associated likelihood ratio.

In this workshop, we will see that if the DNA from a person is considered to be present on an item, there are important considerations that the forensic scientist should make to assist the court in assessing “how” or “when” this DNA was transferred. Because of their specialized knowledge on transfer, persistence, and prevalence of DNA, forensic scientists can and should share this with the court by considering their results in the light of the alleged activities. It should be clear, that the statistical evaluation of a DNA profile result, which contributes in addressing the question regarding “whose DNA is it?” is an entirely different question from “how and when did the DNA get there?” The “how” and “when” questions involve assessing the results while considering transfer and persistence of DNA, as well as considerations of background DNA and sometimes contamination. These issues are dealt with by assessing the biological results in the context of propositions that relate to a contested activity, the so-called Activity Level Propositions.

This workshop will use an interpretation framework and illustrate the difference between subsource and activity propositions, and how one can help address the issues in a case. It will provide guidance as to how a practitioner can present the evidence in a fair, balanced, logical, and transparent way to assist the court in its deliberations. This workshop will highlight that the findings—no matter how robust the results are given subsource level propositions—can potentially be misleading if used to answer activity propositions with vague responses such as “could have,” “it’s possible,” or “maybe.” This workshop will illustrate the traps that arise from conflating one level of proposition with another and outline the need for training in interpretation issues, given there are questions regarding transfer.<sup>1</sup> This workshop recognizes the challenges faced in giving testimony and demonstrates how tools such as Case Assessment and Interpretation and Bayes Nets can help address these challenges.<sup>2,3</sup> Testimony simulations will be presented to illustrate how to avoid classical pitfalls when testifying.

The workshop presenters include academics and specialists who have a wealth of experience in assisting court in complex DNA cases.

#### Reference(s):

1. Cook R., Evett I.W., Jackson G., Jones P.J., Lambert L.A. A hierarchy of propositions: Deciding which level to address in casework. *Science & Justice* 38(4) (1998) 231-239.
2. Cook R., Evett I.W., Jackson G., Jones P.J., Lambert J.A. A model for case assessment and interpretation. *Science & Justice* 1998; 38(3): 151-156.
3. Taylor D. and Champod C. Using Bayesian networks to track DNA movement through complex transfer scenarios. *FSI Genetics* 42 2019 p 69-80.

#### DNA, Testimony, Transfer



### W11 A New Realm of Novel Psychoactive Substance (NPS) Opioids and NPS Benzodiazepines—Analytical and Interpretive Considerations

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**Learning Overview:** After attending this workshop, attendees will be able to: (1) discuss current drug trends for NPS, specifically benzodiazepines and opioids; (2) discuss the difficulties in interpreting NPS benzodiazepines and opioids in Driving Under the Influence of Drugs (DUID), death investigation, and Drug-Facilitated Sexual Assault (DFSA) cases; and (3) assess different instrumentation for the analysis of NPS benzodiazepines and opioids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing updated information on the ever-changing presence of NPS benzodiazepines and opioids. Presentations will cover a variety of topics, including emergence drug trends, pharmacology, analytical methods, case findings, interpretation, NPS combinations, and more.

Accurate and timely identification of NPS are critical for forensic, clinical, public health, and public safety communities. In the past year, the emergence of new NPS benzodiazepines and NPS opioids have challenged the forensic science community, as these substances have been found among drugged driving, sexual assault, and death investigation casework. Most notably, flualprazolam emerged as a new NPS benzodiazepine and has since become the more prevalent substances in its class. The NPS opioid landscape has not been the same since the core structure scheduling of fentanyl-related substances. Fentanyl analogs are substances of the past and new drugs, like isotonitazene and brophine, have become common names. These shifts in NPS markets—the cat and mouse games—have left laboratories scrambling to stay ahead of new substances with quick life cycles of just three to six months. While detection remains an important issue, interpretation of NPS results can be equally as challenging. New NPS benzodiazepines and opioids emerge so quickly there is often no scientific data about activity, potency, and toxicity. In addition, these two classes become exceedingly more challenging when found in combination, allowing for additive Central Nervous System (CNS) depressive effects. Information sharing and discussion about emerging NPS results is essential for forensic scientists to remain aware of new trends, overall impact, and toxicological interpretation.

This workshop will provide information related to the prevalence and distribution of NPS benzodiazepines and NPS opioids in the United States and interpretation of important results. Analytical methods and tools for NPS detection in forensic chemistry and forensic toxicology will be discussed, along with timely results from the analysis of casework specimens, primarily in biological fluids. Additional topics included in the program will focus on trends, history, and pharmacology of these important NPS classes, as well as methodologies for the compilation and distribution of data and findings.

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**NPS, Toxicology, Forensic**



### **W12 Forensic Anthropology Databases for Research Purposes**

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**CANCELED**

## W13 Forensic Multimedia Authentication: Real-Life Problems and Solutions

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**Learning Overview:** After attending this workshop, attendees will: (1) be familiar with the latest developments in forensic video and audio authentication and 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, 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, and audio compression history assessment. It 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.

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 is a video authentication framework, focusing on camera verification/identification and image and video attack detection. This includes a quick overview of a digital video file creation chain for contextual information of the artifacts that influence the final digital media streams based upon the 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 3D scanned models of vehicles can be misused to make geometrically accurate fake video implicating false suspect(s). But it also can be used to photogrammetrically authenticate a make and model of the vehicle captured in the Closed-Circuit Television (CCTV) recording. This workshop will present a novel computational method for video-based Forensic (i.e., with mathematically accurate error estimates) vehicle Make/Model Authentication (FMMA) through Height-Preserving Constraints (FMMA-HPC). Given a sufficient number of identifiable vehicle features-set, and a required 3D vehicle scan, the FMMA-HPC yields an accurate authentication/disparity error measure between the observed image of an unknown vehicle and the conjectured vehicle make and model, as presented in the 3D scan model. This workshop will demonstrate the application of FMMA-HPC technique on several examples. One of the examples comes from expert-witness forensic photogrammetry testimony utilizing an earlier Height Preserving Projection (HPP) method, which photogrammetrically concluded a “Features-Insufficient or 3D Inconsistent video data, for a positive vehicle Make/Model Authentication vs. Merritt Truck 3D Model,” from the evidentiary CCTV video, during the 2019 Charles Merritt death penalty trial in San Bernardino, CA. This scientific public data should be peer-review studied and scrutinized by the forensic science community, using similar and alternative computational methods, given its enormous social implications.

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, Modified Discrete Cosine Transform (MDCT) map, MDCT frame offset analysis, and microphone attribution.

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### Forensic Multimedia Authentication, Deepfake, Deepvoice

### W14 Policing in 2021—Perspectives, Problems, and Potentials

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**Learning Overview:** The goal of this presentation is to present multiple elements of present-day policing, specifically including use of force and biases, highlighting the complex interaction of myriad elements in police use-of-force cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging discussion and fostering objectivity in the pursuit of justice through truth in evidence in cases involving police use of force.

In today's world, the long-standing concerns regarding police operations and use of force have come front and center in the public eye. Very public cases involving police and quasi-law enforcement, including the killings of George Floyd, Ahmaud Arbery, and Breonna Taylor (to name but three of the known 164 African Americans killed by law enforcement in the first eight months of 2020—an average of two people every three days) have sparked public outcry like none before throughout the United States and migrated into other countries. Blacks are over-represented in police-associated homicides and are killed at a rate over 2.5 times higher than are Whites. Social justice movements like *Black Lives Matter* and *Know Their Names* have gained tremendous support as many continue efforts to find some workable reform.

In order to find a solution, one must first understand the problem. The initial step is to understand the scope of the problem and how it affects the population. Investigative journalists have been at the forefront, reminding the citizenry of the lives lost, the damage done, and exposing injustices in the pursuit of truth. There are limitations as to what can be done, as reporters can only work with the information received. In addition, there may exist an anti-media bias with a tendency to shroud a law enforcement agency in a cloak of red tape or behind a wall of bureaucracy.

The disproportionate number of African Americans killed is disturbing and calls for clarification of any real or perceived biases that may impact cases. One example is in Artificial Intelligence (AI) facial comparison software. The practical issues and how these systems function affect outcomes. Data indicate that at present some biases are quite literally built into the system. Digital imaging facial recognition software has proved a useful tool in assisting with the identification of subjects; however, there can be a dramatic discrepancy in reported error rate based on gender and pigmentation. The neural networks used for facial comparison and the training sets used affect the results. If training sets are not balanced, the AI software will have bias depending on the examples it has learned. Failure of vendor software delivery to United States law enforcement end users is another important consideration.

Once a subject is identified, the stage is set for the interaction that too often ends tragically. Law enforcement officers are empowered by law to protect utilizing deadly force, if necessary. The use of force is a continuum predicated on de-escalating and neutralizing perceived threats. The officer must rely on training and experience to make literal life-or-death shoot-or-don't shoot decisions in an instant. These decisions can have life-long repercussions for the subject, the community, and the officer. At the less-than-lethal end of the spectrum are conducted energy weapons, designed to immobilize a subject by means of inducing an involuntary motor response. These weapons have been the subject of controversy, with debates about their safety and potential involvement in at least some deaths. Less-than-lethal does not necessarily mean non-lethal.

In all such cases, the forensic pathologist is called upon to determine, to the extent possible, the mechanism (cause of death) as well as the circumstances surrounding a fatality (manner of death). Some deaths and proffered mechanisms (conducted energy weapons, prone maximal restraint, etc.) are contentious and, regardless of the investigative outcomes, controversy may be anticipated. In some instances, medical decisions may provoke public rage and/or personal attacks. In some medicolegal death investigation systems, this may be compounded when the examiner certifying the death is an elected coroner whose very livelihood may be in the balance when an unpopular medical decision is made.

Ultimately, in instances where police use of force results in serious injury or death, the matter will likely go to civil and/or criminal prosecution. One such case involved the killing of Walter Scott by Officer Michael Slager. Bystander video of part of the incident documented the officer shooting the fleeing subject from behind. The state murder trial ended in a hung jury. Slager was subsequently charged with federal civil rights charges to which he pled guilty in exchange for dropping the state murder charges. He was sentenced to 20 years.

The broad areas of interest involved in a police use-of-force case intersect in one effort to pursue justice through truth in evidence.

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#### Police, Force, Fatal

### W15 STR Wars: The Rise of Sequencing

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**Learning Overview:** After attending this presentation, attendees will have been introduced to forensic DNA sequencing methods that are currently being adopted for the analysis of Combined DNA Index System (CODIS) core Short Tandem Repeat (STR) loci, mitochondrial DNA, autosomal Single Nucleotide Polymorphisms (SNPs), and other non-traditional markers.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by showcasing the innovative sequencing strategies that international forensic laboratories are implementing to improve their approach to forensic casework.

The foundation of modern forensic DNA analysis is STR typing on Capillary Electrophoresis (CE) technology. Using one- to two-dozen highly polymorphic loci in a multiplexed amplification, DNA profiles from any two random individuals can be discriminated with high certainty. STR data are numerically recorded and simple to store in electronic databases. This has positioned STR typing at the center of DNA databasing efforts (e.g., the National DNA Index System [NDIS] and International Criminal Police Organization [INTERPOL]) that bridge law enforcement and government agencies to solve forensic cases. The advent of probabilistic genotyping for STR mixture deconvolution has solidified its role in criminal casework.

Despite the robustness of STR typing, it has fundamental drawbacks that preclude its use from becoming ubiquitous. STR typing with CE technology requires intact nuclear DNA fragments of several hundred base pairs for successful amplification. Therefore, it has limited use for degraded DNA that may be found in aged remains, burned material, or hair shafts. Additionally, the core STR loci are randomly segregating and not revealing of linkage blocks that facilitate extended kinship analysis. As a result, traditional autosomal STR markers limit kinship assessments to close relatives, such as those in a nuclear family.

The gaps in forensic DNA testing that were left by STR typing on CE platforms have been filled in with sequence-based analyses of other genomic targets such as mitochondrial DNA (mtDNA) and nuclear Single Nucleotide Polymorphism (SNP) markers. The detection of these markers allows for identity, ancestry, and phenotype predictions, as well as kinship analyses. Furthermore, the implementation of Massively Parallel Sequencing (MPS) methodologies into forensic laboratories has opened the door to feasible protocols for sequencing large number of these loci. Commercial MPS kits targeting STRs as well as SNPs and the entire mtDNA genome are now available and have been implemented in forensic laboratories for casework. Prototype SNP panels are being developed by the International Commission on Missing Persons and the Visible Attributes Through Genomics (VISAGE) Consortium for kinship assessment and DNA phenotyping, respectively. The increase of the fundamental knowledge on new traits such as facial shape may be incorporated into these newly developed MPS tools. High-density SNP generation through microarray testing and MPS allow for extended kinship analysis and genetic genealogy. However, these advancements cannot be implemented without considering the ethical and legal implications. Issues with method validity, data management, genetic privacy, and lack of regulations/standards must be examined as the field of forensics continues to envelop this powerful technology. Yet, MPS technologies continue to evolve at a rapid pace, and soon the value of MPS may eclipse that of the longstanding CE technology.

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#### DNA, Sequencing, Human Identification

### W16 Technology and Design of Security Documents for Counterfeiting and Alteration Resistance

*Joel A. Zlotnick, MSFS\**, United States Department of State, Washington, DC 20522; *Dennis J. Ryan, MBA, Applied Forensics LLC, East Meadow, NY 11554*

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**Learning Overview:** After attending this workshop, attendees will understand two facets of document security. First, this workshop provides an overview of common document security features, such as security fibers, watermarks, microprinting, color shifting inks, ultraviolet printing, holograms, laser engraving, and many others. The second and equally important subject is how document components can be integrated with one another, and with document artwork, in ways that allow the security value of each anti-counterfeiting technology to be maximized.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing document examiners and other forensic scientists with a better understanding of how security documents are designed and counterfeited, improving the ability of examiners to differentiate between genuine and counterfeit.

Document counterfeiting remains a persistent problem for governments and the private sector alike and has produced an explosion of development in anti-counterfeiting technologies in recent decades. These include innovations in paper and plastic substrates, security inks, specialized printing technologies, holograms and other classes of features used in security documents, such as banknotes, passports, identity cards, and birth records. However, security feature technologies are often regarded as simple checkboxes, where a specific security feature technology is either included or not included in a security document design. In this context, the focus is often limited to ensuring that the document contains some designated minimum quantity of security features. Some attention may also be devoted to how various features fight against counterfeiting and/or alteration, but usually only within the limited scope of the feature's primary function. Certainly, technology selection is an important first step and does have broad consequences with regard to a security document's cost and manufacturability. However, security features are not integrated in the same way in every unique document or by each document issuer, so not all implementations of security feature technologies are equally effective.

Specifically, security feature technology selection alone does not account for how security design strategies can make or break the value of a technology and, by extension, the security of the document. Thoughtful and purposeful use of security feature technologies can maximize their effectiveness by integrating them with other document components, facilitating easier inspection by document users, or allowing each feature to generate additional value by occupying ancillary security roles that extend its functionality. For example, watermarks are a foundational security feature that have been used to protect paper substrates from counterfeiting for hundreds of years. Yet many modern watermark implementations allow a watermark to fill not only its primary role of deterring substrate counterfeiting, but also new roles as an anti-alteration feature. Other watermarks occupy unprinted areas of paper to signal document users that a watermark is present, and further use the watermark design to communicate specific information about the document issuer or purpose, both of which contribute to the ergonomic accessibility of the feature. Similar thinking can be extended to many other security feature technologies, such that the utility of each can be maximized with the help of specific design strategies relevant to each particular technology. In contrast, poor security feature implementations can waste resources, create a false sense of security, confuse document users, or even expose the document to counterfeiting or alteration risks.

This workshop will provide attendees with a two-sided view of security document design. First, attendees will learn about contemporary innovations to both old and new security feature technologies, and design strategies, in use in contemporary security documents. Second, attendees will understand how innovations in genuine document manufacturing affect counterfeiter perceptions, counterfeiter imaging and printing workflows, counterfeiting costs, and how and where counterfeiters can be forced to compromise on counterfeit quality and manufacturability. Virtual hands-on exercises will be facilitated by attendees examining their own personal passports, driver's licenses, birth records, and other security documents.

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#### Counterfeit, Printing, Fraud



### W17 An Update on Analytical Approaches for Hemp/Marijuana Differentiation

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**Learning Overview:** After attending this presentation, attendees will understand the analytical schemes that are being employed to differentiate hemp from marijuana and will gain insight from laboratories that have implemented new methodology to address this issue.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing additional insight regarding the use of various analytical schemes and the use of decision-point assays for the differentiation of hemp from marijuana across jurisdictions in federal, state, and private sector laboratories.

During this workshop, analytical approaches for the differentiation of hemp from marijuana will be presented. Following the passage of the Federal Farm Bill and the implementation of new methodology, laboratories will share their analytical findings and discuss various challenges associated with different approaches.

The pursuit of hemp as an agricultural commodity has placed a significant burden on laboratories that are now required to implement new methodology to address this issue. The 2018 Federal Farm Bill established a threshold of no more than 0.3%  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) on a dry weight basis for *Cannabis sativa L.* plants, derivatives, or extracts. The United States Drug Enforcement Administration was the first to deploy an analytical scheme that utilized an administrative threshold for the differentiation of hemp from marijuana in plant material. This approach, which has been successfully utilized and accepted in the courts in other forensic disciplines (e.g., toxicology drug screening) for decades, permits the use of a qualitative assay using a specified (legislative or administrative) threshold or decision-point.

During this workshop, operational laboratories will share their experience using various analytical approaches, ranging from decision-point assays (using various thresholds) to full quantitation. Focusing on the analysis of plant material, analytical schemes being deployed across the United States will be presented following a 2020 survey conducted by the Organization of Scientific Area Committees (OSAC) Seized Drugs Subcommittee. Federal, state, and private sector laboratories will share their experience and analytical findings following the deployment of new methodology. Strategies for implementation and analytical challenges will be addressed. The jurisdictional influences guiding the various approaches and their impact on the development of consensus-based standards for the identification and reporting of marijuana will be discussed.

Changes in implementation strategies to allow for future adaption to other matrices (e.g., oils/liquids) will be addressed. An overview of the National Institute of Standards and Technology (NIST) Cannabis Quality Assurance Program (CannaQAP), aimed at helping forensic laboratories demonstrate and improve measurement comparability and competence, will be provided. Findings and lessons learned from the first CannaQAP exercise will be discussed and the importance of interlaboratory comparisons and collaboration will be emphasized.

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#### Hemp, Marijuana, Cannabis



### W18 Competency to Stand Trial: Nuances in Competency Assessments

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**Learning Overview:** The goal of this presentation is for attendees to first understand the basic requirements of an assessment for competency to stand trial. From there, attendees will build skills to identify the challenges in assessments and techniques used to address them. This workshop will explore competency assessments through case studies and give attendees the opportunity to apply what they have learned at the end with case examples and discussions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing instruction to both improve performance and understanding of competency to stand trial assessments, which will ultimately improve case outcomes.

Competency to stand trial assessments can impact the entire course of a legal case. While not every defendant will undergo one of these assessments, it is important for their legal team to understand when to order one and how to interpret the assessments they receive in order to best serve their clients. Competency assessments are nuanced and the art of the assessment may not be well understood by those outside of forensic psychiatry. This workshop includes discussion of several topics related to competency to stand trial. Starting with the history of assessments and their utility in legal arenas, this workshop will explain the basics of competency assessments, details of the evaluations, testamentary capacity, and information that could be provided by attorneys to facilitate the evaluation. Considerations on how to proceed when a defendant refuses to participate and/or concerns about feigned incompetence will also be discussed.

This workshop will also include information on the symptoms of psychiatric diagnoses that can have a negative impact on a defendant's competency, challenges in assessing competency, and special topics to include sovereign citizens and other unique presentations that may face attorneys and forensic evaluators alike. Case examples will be provided to highlight these special issues and considerations. This workshop's target audience includes both forensic evaluators and attorneys as the defendant's legal team and the forensic evaluators should have an understanding of each other's needs and concerns regarding competence in order to move cases forward in the legal process.

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#### Competency, Psychiatric, Assessment

### W19 Most Valuable Publications (MVPs) of Forensic DNA: Examining the Most Valuable Publications in the Field

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**Learning Overview:** After attending this workshop, attendees will have gained an understanding of the principles involved in DNA analysis and interpretation, knowledge of core foundational literature supporting these principles, and information that can strengthen training programs for DNA analysts.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by contributing to the creation of a defined body of knowledge covering historical and foundational literature, published validation studies, and recent, relevant publications that can fortify reliable forensic DNA analysis and interpretation.

Effective training and continuing education are crucial to keep up with evolving forensic DNA technologies and applications. Forensic laboratories invest in the continuing education of their staff. Universities with forensic science programs seek to prepare their students to be future contributing caseworkers. Stakeholders in the criminal justice system (e.g., law enforcement personnel, lawyers, and judges) using DNA results also benefit from regular training and continuing education to understand capabilities and limitations of methods and practices. Forensic scientists, students, and stakeholders can profit from drawing from a common informative knowledge base.

Thousands of publications in dozens of peer-reviewed journals exist on the topic of forensic DNA. This ever-growing body of scientific literature becomes increasingly challenging to monitor, much less incorporate into forensic laboratory training programs. For forensic scientists in the trenches working cases, understanding which research publications are most informative would be helpful. DNA technical leaders and analysts could benefit from receiving regular updates on relevant and available articles and the creation of lists of relevant articles in specific areas of interest to forensic DNA casework. This workshop will introduce attendees to an initial effort to identify and describe the MVPs in the field.

The National Institute of Standards and Technology (NIST) has been congressionally funded to perform scientific foundation reviews of select forensic disciplines. These reviews are intended to identify and document what is well known and well supported empirically in a forensic field and identify gaps that need further study. Over the past several years, the abundant literature on DNA mixture interpretation, which is the initial NIST foundation review, has been gathered and examined. An important goal of this effort is to identify, consolidate, and share core principles and supporting publications with the community to encourage deeper learning and understanding of forensic DNA.

Based in part from the NIST review and in discussions with experienced forensic DNA scientists, an initial list of 400 informative publications has identified across 26 topic categories in forensic DNA. This list builds upon references cited in the July 2020 Scientific Working Group on DNA Analysis Methods (SWGDM) Training Guidelines and efforts underway within the Organization of Scientific Area Committees for Forensic Science (OSAC) Biology/DNA section.<sup>1</sup> The information learned in this workshop will support published training standards from the AAFS Standards Board (ASB) DNA Consensus Body and on the OSAC Registry as well as professional development described in section 16 of the recently updated Federal Bureau of Investigation (FBI) Quality Assurance Standards.<sup>2-4</sup>

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#### Forensic DNA, Scientific Literature, Training Standards

## A1 The Introduction of a Cranial Gunshot Trauma Photographic Atlas

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**Learning Overview:** After attending this presentation, attendees will be familiar with a new resource to be utilized in forensic gunshot analyses. This novel Cranial Gunshot Trauma Photographic Atlas presents a photographic series of 45 human crania that have been subjected to ballistic trauma under controlled conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of the competence and performance of attendees by the availability of a Cranial Gunshot Trauma Photographic Atlas that can assist with understanding a baseline of damage to be expected from a specific shooting scenario. This will assist practitioners in analyzing gunshot trauma from unknown forensic scenarios by increasing the knowledge of how extrinsic variables affect patterns of gunshot trauma.

The Cranial Gunshot Trauma Photographic Atlas is a uniform series of professional photographs that were taken following controlled gunshot experiments to 45 donated human heads. While high-quality photos of cranial gunshot trauma are found in books and scientific articles, these images are limited in scope and are often from instances of gunshot trauma where certain extrinsic and intrinsic variables are unknown. The Atlas introduced here highlights the patterns that exist in cranial gunshot trauma when certain variables are controlled.

The controlled shooting experiment was designed to test the effects of bullet construction on cranial gunshot trauma patterns. To do this, the experiment controlled for extrinsic variables, including weapon, shooter, distance, caliber, and bullet velocity. Donated, fleshed human heads ( $n=45$ ; 23 females, 22 males; age at death 54–90 years) were shot once by the same expert marksman with a Smith and Wesson® model 438 J-frame revolver with a 1 $\frac{7}{8}$ " barrel. The ammunition used was .38 Special Winchester® Train and Defend™ in both full metal jacket and jacketed hollow point constructions. Each round weighed 130 grains; this allowed control for differences in resultant trauma due to bullet mass and to instead isolate the effects of bullet construction. The shots were all from a distance of three yards and either from an anterior direction through the frontal bone or from a lateral direction through either the temporal bone or parietal bone. A ballistic chronograph was used to record the impact velocity of each shot, and a fleece backstop was in place behind the head to contain bullets from perforating shots. Post-shooting, all heads were sectioned to allow for endocranial observation, then macerated using standard methods.

Within the Atlas, each individual skull is depicted by professional photographs, including, but not limited to: entrance and exit wounds from both ectocranial and endocranial views; a 360-degree rotational series of the cranium; specific views of gunshot-related fractures; and any present pathology unrelated to gunshot trauma. The known ages and sexes of each individual are listed along with bullet construction and velocity data. All skulls were scanned for bone mineral density, and these values are also included in the Atlas. Including these data is intended to allow practitioners to observe how factors such as shot location, age, sex, and bone mineral density affect patterns of gunshot trauma. The anticipated value of this Atlas will be in its comparative strength for practitioners who are faced with analyzing gunshot trauma with unknown extrinsic factors.

The Cranial Gunshot Trauma Photographic Atlas is available for forensic practitioners by request and is in a PDF file format to allow for ease of use. There is also potential to expand the Atlas by adding photographs from any future controlled gunshot studies and continuing to further the biomechanical understanding of cranial gunshot trauma.

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### Ballistics, Gunshot Trauma Analysis, Forensic Anthropology

## A2 Fusion and Fracture: Forensic Implications of the Hyoid Bone

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**Learning Overview:** The goal of this presentation is to inform attendees of the relationship between the state of fusion in the hyoid bone and fracture patterns with regard to implications for autopsy practices.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the thyroid cartilage and associated soft tissue are better indicators of trauma to the neck than the hyoid bone.

The structures of the neck often provide critical clues in determination of cause and manner of death in a forensic autopsy. Trauma to the hyoid bone has long been considered indicative of injury to the neck, and careful dissection and examination of the hyoid bone and laryngeal cartilages, along with overlying musculature and soft tissue, is a standard part of the autopsy examination. The hyoid bone and laryngeal cartilages fuse and ossify at variable rates, however, which may impact their susceptibility to fracture and the subsequent interpretations of trauma by pathologists and anthropologists.

This study examines the fusion of the body of the hyoid to the greater cornua along with the associated laryngeal cartilages in individuals submitted for examination post-autopsy in order to determine whether hyoid fusion is significantly correlated with evidence of trauma in the neck by asking: (1) whether fusion is related to risk of fracture, and (2) whether absence of fracture means absence of trauma. Further, the study examines the relationship between manifestations of trauma in the hyoid and those in the laryngeal cartilages to determine whether one structure is a better indicator of neck injury than the other.

The neck organs of 123 individuals (53 females, 70 males) removed at autopsy by forensic pathologists were photographed and macerated in the Forensic Anthropology Laboratory. Evidence of neck hemorrhage in the soft tissue was noted, as were suspected circumstances of injury (e.g., ligature strangulation, blunt force trauma). Factors potentially contributing to variation in rates of ossification/fusion and fracture patterns such as age at death and toxicology were also noted in the analysis.

Within the current sample, state of fusion of the hyoid bone is unrelated to sex. State of fusion is, however, dependent on age (Fisher's exact test with 10,000 replicants,  $p$ -value=0.007). These results demonstrate that fracture of the hyoid bone is related to its state of fusion, with unfused hyoid bones exhibiting fewer incidences of fracture than those with either unilateral or bilateral fusion ( $\chi^2=7.077$ ,  $df=2$ ,  $p$ -value=0.029). Fracture of the hyoid bone is independent of hemorrhage or bruising within the soft tissues overlying the larynx. In contrast, hemorrhage or bruising of the overlying soft tissues of the neck is significantly associated with damage to the laryngeal cartilages (Fisher's exact test,  $p$ -value= $2.42e^{-04}$ ).

These results suggest that the absence of trauma to the hyoid bone cannot be used to rule out strangulation or other forms of injury to the neck. Additionally, hyoid fracture is not statistically related to the presence of a ligature. In fact, only 8 of the 25 cases in which a ligature was present (32%) exhibited a fracture of the hyoid. The presence of a ligature is significantly related to the presence of hemorrhage or bruising in the soft tissue (Fisher's exact test,  $p$ -value=0.021), where 84% of the cases involving a ligature also exhibited bruising or hemorrhage. The presence of a ligature is even more strongly related to the presence of injuries to the laryngeal cartilage (Fisher's exact test,  $p$ -value=0.001), where 80% of the cases involving a ligature also involved damage to the cartilage.

The results of this study indicate that, when present, the laryngeal cartilages are a much better indicator of trauma to the neck than the hyoid bone as the state of fusion of the hyoid bone is directly related to the manifestation of trauma in that structure. Forensic pathologists suspicious of trauma to the neck are encouraged to remove the neck *en bloc*, without "opening" it, in order to allow for a complete dissection or maceration of the hyoid and laryngeal cartilages in order to fully document traumatic injury.

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### Hyoid Fusion, Trauma in Neck, Fracture Patterns

## A3 The Frequency and Pattern of Cardiopulmonary Resuscitation (CPR)-Related Fractures in an Infant Autopsy Sample

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**Learning Overview:** The goal of this presentation is to evaluate the frequency of rib fractures related to CPR in an infant autopsy sample and to describe the observed pattern, including posterior rib fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the frequency of CPR-related rib fractures in an infant autopsy sample and describe the general pattern of CPR-related rib fractures, which is potentially valuable for forensic practitioners in differentiating therapeutic intervention from inflicted injuries.

Recent pediatric literature describes the occurrence of pediatric rib fractures associated with CPR as uncommon or rare.<sup>1,2</sup> Two reasons why these studies may not be applicable to infant autopsy samples include: (1) the majority of research is based on radiographic studies, although acute rib fractures are notoriously difficult to identify radiographically; and (2) many of these studies were conducted prior to 2000 when the one-handed (or two-finger) CPR technique was recommended for infants.<sup>3</sup> In 2000, the preferred technique for infant CPR was modified to the two-thumb technique when performed by two medical personnel.<sup>4</sup> The current study builds on previous research by evaluating rib fracture data from a narrowly defined subset of the infants in the Infant Injury Database (IID).<sup>3</sup>

Based on published research, CPR-related rib fractures were hypothesized to occur most frequently on the anterior region of the ribs.<sup>5</sup> It was also hypothesized, based on years of observation, that CPR-related rib fractures would: (1) occur more often on the left side than the right; (2) frequently occur on the posterior region; and (3) occur more frequently in younger infants. Non-parametric statistics and frequency data were used to evaluate these hypotheses and examine the distribution of CPR-related rib fractures.

The study sample consisted of 387 infants ( $\leq 12$  mos. of age) autopsied at the Harris County Institute of Forensic Sciences from 2010 to 2020. All cases with skeletal injuries other than acute rib fractures, and cases with a cause of death classified as undetermined or trauma-related, were excluded. Manner/cause of death categories for these infants included undetermined/co-sleeping, undetermined/sudden unexplained death in infancy, natural/sudden infant death syndrome, natural/other (various causes of death related to natural disease), and accident/asphyxia or drowning. All infants had documented CPR and received a complete rib examination post-autopsy by a forensic anthropologist. CPR fractures were diagnosed in the context of preceding history lacking trauma and a predisposition to fracture, and the following fracture characteristics: acute fracture without signs of healing or inflammatory response, little to no periosteal hemorrhage, and primarily located in the anterior and/or anterolateral rib segments. Region of rib fracture was documented according to Love et al.<sup>6</sup>

Acute rib fractures likely associated with CPR were observed in 182 infants (47%). Infants with rib fractures were significantly younger than the infants without rib fractures ( $U = 14,747, p < .001$ ). Eighty-five percent of the infants with rib fractures were between 0 and 4 months of age, with 1-month-old infants accounting for the greatest proportion of cases (~32%). Regarding infants with CPR-related rib fractures, there was a weak negative correlation between age and number of rib fractures ( $r_s = -.22, p = .002$ ). The number of left-sided rib fractures was significantly greater than right-sided rib fractures ( $Z = -3.64, p < .001$ ). Approximately 93% of rib fractures occurred on ribs 2–6, primarily in the anterior region (61%), followed by the posterior (~20%), anterolateral (~14%), and posterolateral (~2%). Anterior rib fractures occurred on ribs 1–9, with ~88% observed on ribs 2–5. Posterior rib fractures occurred on ribs 1–9, with ~80% occurring on ribs 2–6. Anterolateral rib fractures occurred on ribs 1–7, with ~96% occurring on ribs 2–6. Chi-square tests indicated a significant negative association between the occurrence of left anterior fractures and left posterior fractures ( $\chi^2 = 18.34, p < .001$ ) and left anterior fractures and right posterior fractures ( $\chi^2 = 15.04, p < .001$ ).

These findings indicate that CPR-related rib fractures occur significantly more often in younger infants, and, when present, are more numerous in younger infants. CPR-related fractures occurred primarily on ribs 2–6, more frequently on the left side than the right, and on the anterior region. While posterior rib fractures occur less frequently than anterior fractures, their occurrence is frequent enough to be significantly associated with left anterior rib fractures.

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### Forensic Anthropology, Pediatric, Cardiopulmonary Resuscitation

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## A4 A Random Forest Approach to False Start Analysis

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**Learning Overview:** The goal of this presentation is to inform attendees of the accurate prediction capability of regression random forest models for false start data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a novel method for saw mark analysis that is complementary to current methods.

**Introduction:** In an attempt to increase objectivity in forensic disciplines, statistical and quantitative analyses are used to complement traditional techniques. Micro-Computed Tomography (micro-CT) of false starts allows these devices to be applied to the forensic toolmark field, which commonly still utilizes human evaluation techniques. Micro-CT is an ideal method for quantitative analysis as measurements may be taken from the digital model without altering the sample during the evaluation. Furthermore, as micro-CT is non-destructive, traditional evaluation methods can be used complementarily.

**Method:** Three hundred forty experimental false starts were created on fleshed human cadaveric long bones to represent the variability of marks seen in forensic casework. A total of 38 saws, of multiple classes spanning hand saws, hacksaws, and power saws, were used. Micro-CT scans of each bone were taken and cross-sections of the false starts collected to generate a 2D profile image. Quantitative data, in the form of seven distinct measurements, were established from the cross-sections. The seven measurements used aimed to describe the false start profile quantitatively, and included three width measurements, convex height, and three profile floor angles. Two regression random forest models were built on the experimental data with the aim of predicting the saw blade thicknesses from the false start measurements. An Unknown Class Model (UCM) was created from one regression random forest built on the pooled experimental measurement dataset. A Known Class Model (KCM) was built on the data separated by class and hence consisted of three regression random forests. A second set of experimental false starts was similarly created and measured, but excluded from the training data, to test the accuracy of the random forest models.

**Results:** Micro-CT is a suitable tool for the quantitative analysis of false starts on bone. Regression random forest models can be successfully created and are able to predict the blade thickness of the saw used to create a false start with up to 100% accuracy, within two standard deviations of the measured mean, if the saw is of a known class. If the class of the saw is unknown, saw blade thickness prediction is less accurate, with 88.3% of thicknesses being correctly predicted to within three standard deviations of the measured mean.

**Conclusion:** Random forest models have been produced from experimental false start measurement data to accurately predict saw blade thicknesses quantitatively. Knowing the class of the saw enables a more accurate prediction of saw blade thickness from false start measurements. Application of this technique in combination with traditional analysis techniques may provide further information to toolmark analyses in casework.

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### Toolmark, False Start, Statistical Analysis

## A5 Revisiting Serrated vs. Non-Serrated Kerf Marks in Sharp Force Trauma (SFT) Analyses

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**Learning Overview:** The aim of this presentation is to inform attendees of the questionable utility of the qualitative method of analyzing morphological characteristics of kerfs as well as the quantitative method of measuring length and width of kerf to positively infer the causative implement.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the need to develop more robust methodologies to identify blade types to ensure reproducibility.

Sharp Force Trauma (SFT) is one of the leading types of injury in the United Kingdom. As of March 2020, injuries caused by knives or other sharp implements have been implicated in 39% of all homicide cases. However, much of SFT research has focused on saw and hacking marks related to dismemberments. These events are arguably much less common than stab injuries, which have received little attention in research. Therefore, this study was designed to investigate stab injuries through a combination of quantitative and qualitative methods. This study adopted previously established methods for distinguishing morphological characteristics of “serrated” and “non-serrated” knife blades. It sought to examine whether: (1) the kerf (marks left by implements) characteristics and striation patterns can infer the causative implement in stab injuries, and whether (2) the metric analysis provides meaningful insights in differentiating the blade types.

To address these questions, knives with serrated and non-serrated blades were utilized. These were mounted onto a partially mechanized guillotine fashioned from a paper-cutter machine mimicking a cantilever system. The purpose of this mechanistic effect was to mitigate possible variations of the penetrative angle of the knife while maintaining reasonably consistent human force when delivering the stab marks. *Sus scrofa* (pig) specimens ( $n=30$ ) chosen in this study were macerated and fully skeletonized to avoid possible inter-variations in soft-tissue depth between each sample. These stab marks were then visually observed to record kerf characteristics resulting from each blade type. Two-dimensional images were also obtained using a Scanning Electron Microscope (SEM) to examine the microscopic striations, and these images were later processed through the program ImageJ to obtain metric measurements of the lengths and widths of the kerfs. A pilot study with intra- and inter-error tests was conducted by enlisting three individuals outside of the anthropology field to measure the lengths and widths of the kerfs. This was to ensure that the measurement protocols established in this study were consistent with minimal vagaries and were reliable.

The qualitative analysis in this study found that macroscopic and microscopic characteristics previously associated with serrated and non-serrated blades were not reproducible. The morphological features resulting from each blade type were either not produced or occurred at a much lower rate than expected to infer the type of causative implement. Further, the high-resolution imaging technique provided by SEM could not observe the characteristic striation patterns associated with either blade type on most of the sample specimens. In terms of quantitative analysis, the one-way Analysis of Variance (ANOVA) ( $p$ -value $<0.05$ ) showed that there was a statistically significant difference between lengths and widths between serrated and non-serrated blades. However, this does not provide a meaningful context in identification without reliable qualitative features to corroborate the metric analysis. This indicates that there is a crucial need to develop more robust methodologies in identifying blade types that would be in line with current efforts in the discipline of forensic science to ensure reproducibility and scientific rigor. This concerted effort could help avoid the potential for misclassification of knife blades in forensic investigations.

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**Forensic Anthropology, Sharp Force Trauma, Stab Injuries**





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## A6 A Survey of Skeletal Trauma Within the Southeast Texas Applied Forensic Science Collection: A Wealth of Research Opportunity

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**Learning Overview:** After attending this presentation, attendees will better understand the wide range of skeletal trauma represented within the skeletal collection at the Southeast Texas Applied Forensic Science (STAFS) facility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the opportunities available for multidisciplinary forensic research at STAFS.

The STAFS facility is a willied body donor program located in Huntsville, TX, under the direction of the Department of Forensic Sciences at Sam Houston State University (SHSU). STAFS aims to advance forensic knowledge and practice by providing cadavers for research in forensic, anthropological, geological, chemical, and biological sciences, while also offering education and training to internal and external agencies. The facility has received 636 willied body donations since it opened in 2009, with an average of 53 donations per year. STAFS currently operates both outdoor and indoor research facilities. The two-acre outdoor facility provides numerous research opportunities to recreate forensically relevant scenarios and perform taphonomic and other forensic analyses of human remains during the decomposition process. The indoor facility is a Biosafety Level 2 (BSL2) laboratory and includes an intake area, donor storage, necropsy suite for autopsy or maceration, temperature-controlled skeletal collection room, X-ray equipment, digitizer, microtome, and blunt force trauma simulator. The collection currently holds 337 skeletons obtained through the donor program with family consent. The skeletal collection consists primarily of individuals of European descent (83%), with smaller proportions representing African (5.5%), Hispanic (4.4%), and Asian, Middle Eastern, or Native American (1.5%) ancestries. Additionally, the skeletal collection offers opportunities to study the many skeletal pathologies, anomalies, and traumas represented in the collection.

This current project involves surveying the extent of skeletal trauma represented in the collection. Cranial and postcranial elements have been evaluated, with particular attention to those areas that are forensically significant (i.e., head, thorax, and long bones). Each specimen exhibiting perimortem skeletal trauma has been documented, photographed, and categorized (blunt force trauma [ $n=40$ ], gunshot trauma [ $n=30$ ], and sharp force trauma [ $n=10$ ]). By exploring a variety of 2D and 3D imaging modalities, including radiography, photogrammetry, surface scanning, and computed tomography, this project will report on methods for precise imaging, measurement, and documentation of skeletal trauma. The data from this preliminary study will then form the basis for future controlled cadaver trauma studies at STAFS.

In conclusion, the STAFS facility offers broad potential for research and training to advance the application of anthropology, entomology, microbiology, chemistry, geology, and forensic science to medicolegal death investigation.

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### Outdoor Research, Skeletal Collection, Skeletal Trauma

## A7 Homicide Fracture Pattern Frequencies by Sex and Geographic Location

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**Learning Overview:** After attending this presentation, attendees will understand how analyzing skeletal trauma should not be independent of an individual's demographics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the practitioner's knowledge regarding common injury location patterns of homicide victims. By documenting and subsequently exposing normal patterns, one can simultaneously recognize abnormal patterns.

Analyzing skeletal fractures associated with violent deaths is generally conducted independent of population, sex, ancestry, or geographic location. However, injury patterns, or the locations where fractures occur on an individual, can differ dependent on these demographic variables. This study is an analysis of individuals with a manner of death of homicide from two geographically diverse Medical Examiner's Offices (MEO). The MEOs are located in New York, NY, and Reno, NV, further differing in terms of one being metropolitan and the other encompassing largely rural areas. Along with a manner of death classified as a homicide, all individuals had a cause of death associated with traumatic injuries, such as blunt force trauma, gunshot wounds, and sharp force trauma. All skeletal fracture locations were recorded per bone with associated demographic data and cause of death information. Previous research has shown that males and females exhibit varying fracture patterns. Therefore, the sample was separated by sex for all analyses to better elucidate patterns in injury locations. Frequency distributions were created to analyze the relationship between common fracture location and all recorded demographic variables; chi-square tests were performed to test the significance of the relationships.

There were 52 individuals that fit the criteria from Nevada and 76 from New York for a total sample of 128 individuals. When comparing by regions, there is a clear difference in the frequencies associated with cause of death. In the more rural population location, the most common cause of death was gunshot wounds (80%), whereas in the metropolitan area the most common cause of death was sharp force trauma (40.6%). The most common fracture location for both regions was the head (metro 34.3%, rural 46.1%).

Males from geographically diverse areas most frequently incurred fractures to the ribs (rural 80%, metro 37.2%). Females from both MEOs also exhibited a similar trend in injury locations across both samples, with the highest frequency of fractures to the head region (46.1% metro, 80% rural). In contrast to the males, females had comparatively less trauma to the rest of the body.

The results of this study demonstrate that skeletal injury locations are similar within the sexes, regardless of geographic location and cause of death. Interestingly, even though the injury patterns were similar in similar anatomical locations, each geographic location had unique causes of death. Knowing this information provides a foundation for the expected injury types and locations and therefore facilitates understanding overall trauma for an individual. Furthermore, future trauma research should consider differences that may be occurring based on demographics and make an effort to create samples that are inclusive of demographic variables to capture possible variations in cause of death or injury patterns.

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### Trauma Analysis, Homicide, Frequency Tables

## A8 Patterning of Projectile Trauma Associated With a Historic World War II Battlefield Site

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of projectile skeletal trauma patterns associated with historic military contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the pattern and extent of projectile trauma associated with historic battlefield deaths.

Since World War II (WWII), it is estimated that approximately 70% of battlefield wounds are due to explosive munitions, whereas gunshot injuries are more common during “theatre entry operations” in the early phases of a conflict. Clinical literature demonstrates most battlefield injuries affect the extremities, with the extent of the injury determined by the amount of energy transferred, which is dictated by the type of tissue being penetrated and the surface area of the projectile. Although literature is available for current-day military operations, data sources for injuries patterns of WWII cases are not as readily available. A study of the trauma documented by the Defense POW/MIA Accounting Agency’s (DPAA) scientists during their analyses to identify unaccounted-for service members from the Battle of Tarawa provides a unique opportunity to understand injury patterns associated with an amphibious landing assault during WWII and how it compares to modern-day battlefield contexts.

This study utilized a sample of 33 WWII-era casualties where the historic documentation noted the cause of death as gunshot wound(s) and the skeletal material was available for review. All individuals in this sample were associated with the same unit and were killed during a single offensive during the Battle of Tarawa. Some preservation issues prevented a definitive assessment of projectile trauma of the thorax, but nearly all other trauma was readily discernable for these cases. All trauma was documented in accordance with the DPAA-Laboratory’s standard operating procedures, including applicable photography. The type and extent of trauma was tabulated by general body region to produce descriptive statistics of elements effected and to provide an overall pattern of skeletal defects observed. Extreme perimortem and postmortem fragmentation of several elements complicated efforts to assess if a given injury was the result of ballistic or another high-energy projectile; however, the high degree of fragmentation (20+ fragments) was overcome in most cases through reconstruction of the defect to allow for more accurate documentation and interpretation of the projectile trauma.

Over 90% of individuals in the sample display at least one traumatic lesion on the skeleton. Approximately 70% of individuals exhibit some form of trauma to the skull or neck, several exhibiting multiple defects, with approximately 55% of individuals having sustained at least one traumatic lesion consistent with projectile trauma. Almost 40% of cases have at least one projectile or possible projectile defect located on the upper appendages, while only 12% of cases have at least one defect on the lower appendages that is clearly related to projectile trauma. Trauma to the thorax, including ribs, thoracic and lumbar vertebrae, scapulae and clavicaulae, was observed in almost 30% of cases, with the majority of these (~27% of total cases) exhibiting at least one projectile or possible projectile defect. Traumatic lesions were least frequently observed on elements comprising the pelvis, with only 6% of the sample showing at least one defect from possible projectile trauma. The projectile trauma that was readily discernable had a presentation consistent with the prevailing literature for cranial and tubular bone, which allowed for further interpretation on impact trajectory. For the cranium and long bones, 35 distinct defects were identified, with directionality able to be estimated in ~80% of cases.

The overall pattern of trauma, with more injuries to the head and neck as opposed to the extremities, is discordant with modern day clinical data indicating a high percentage of wounds to the extremities in battlefield injuries. Of note, there is a larger percentage of clear gunshot wounds to the skeletons in this sample, which corresponds with expectations in theatre entry operations for current-day military operations. Exploring these similarities and differences, such as demonstrated here, has the potential to improve forensic practitioners’ abilities to interpret trauma from historic battlefield contexts.

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### Projectile Trauma, Tarawa, World War II

## A9 Communist Human Rights Violations in Poland (1944–1956): A Forensic Anthropological Perspective

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of pursuing justice through scientific evidence with no cultural, political, or social bias.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting evidence from two sites that contained the remains of executed Polish citizens during the Communist era.

The establishment of the most probable cause of death on skeletal remains is one of the crucial features determined by forensic anthropologists in the examination process of human remains. Numerous studies from recent years found that forensic examination of skeletal material from the past can reveal essential pieces of information about the history of conflicts and also help to identify the victims.

In recent years, the Institute of National Remembrance carried out some excavation work concerning the victims of the communist regime in Poland. After World War II, Poland and other Eastern Europe countries were under the influence of the Soviet Union. Many citizens were arrested and sentenced to death, including members of underground organizations who fought against the Soviets, the pro-Soviet government, and the secret police.

This study analyzed the results of two excavations conducted in Warsaw and Wrocław. The excavations were a part of the Institute of National Remembrance project named “The search for unknown burial places of victims of communist terror in Poland in the years 1944–56.” A medical and anthropological examination followed the exhumation of the former prisoners’ skeletons.

In Powązki Cemetery in Warsaw, the examination established the presence of gunshot wounds on the bone in 78 out of 194 cases.<sup>1</sup> In Osobowicki Cemetery in Wrocław, Szleszkowski et al. observed the gunshot wounds in 39 out of 223 skeletons.<sup>2</sup> A chi-square test of independence revealed a statistically significant association between the presence of gunshot wounds and the cemetery from where the remains were recovered,  $\chi^2(1) = 26.525$ ,  $p < 0.005$ . All of the statistical analyses were performed in the IBM® Statistical Package for the Social Sciences (SPSS) Statistics 26.0 software.

The fall of communism in Poland in 1989 created an opportunity to study crimes from the past using forensic archaeology and anthropology based on scientific evidence without any bias or pressure. In addition, the forensic examination of the skeletons revealed that the firing squad killed the prisoners sentenced to death in a different way than the one described by communist authorities.<sup>3</sup>

The goal of this research is to identify the differences in the manner of execution between Warsaw and Wrocław. Furthermore, this study demonstrates how forensic anthropological examination can reveal various types of gunshot trauma on skeletons exhumed more than 70 years after death.

This present research brings to light the communist crimes in Poland after World War II and emphasizes how the application of forensic science can restore the truth about crimes in a historical context. This is especially relevant in the present day, where there is an increased polarization of political ideologies.

### Reference(s):

1. Szleszkowski, Ł., Thannhäuser, A., Szwagrzyk, K., and Jurek, T. The possibility of establishing causes of death on the basis of the exhumed remains of prisoners executed during the communist regime in Poland: the exhumations at Powązki Military Cemetery in Warsaw. *International journal of legal medicine*. 2015; 129(4): 801-806.
2. Szleszkowski, Ł., Thannhäuser, A., Szwagrzyk, K., Konczewski, P., Kawecki, J., and Świątek, B. Exhumation research concerning the victims of political repressions in 1945–1956 in Poland: A new direction in forensic medicine. *Forensic science international*. 2014 235, 103-e1.
3. Szleszkowski, Ł., Thannhäuser, A., Kawecki, J., Szwagrzyk, K., and Świątek, B. Reconstruction of methods of execution of the death penalty by shooting in the years 1949–1954 based on exhumation research of “prison fields” in Osobowicki Cemetery in Wrocław. Part II—Analysis of gunshot injuries and an attempt at reconstructing the course of execution. *Archiwum medycyny sądowej i kryminologii*. 2012; 62(2): 87-97.

### Forensic Anthropology, Gunshot Wounds, Communism Victims

## A10 Sex Estimation of a Croatian Population Based on Computed Tomography (CT) Scans of the Crania

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**Learning Overview:** The goal of this presentation is to explain the possibilities of sex estimation (in the Croatian population) based on morphological and metric analysis of orbital dimensions from CT scans.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by rethinking how morphological and metric traits in sex estimation can perform differently in different skeletal elements.

**Aims:** To determine the reliability of sex estimation on the CT scans on the Croatian population in the orbital area.

**Methods:** This study was approved by the Hospital's Ethics Committee and the Ethics Committee of the University Department of Forensic Sciences. A total of 200 CT scans (equal number of males and females, aged 18 years and older) were collected; CT's were anonymized, and only sex and age were disclosed. Only those scans that had intact orbital margins were included in this study, as well as those that had no pathological and traumatic changes that could affect the measurements. Data analysis was performed using the OsiriX® Program OsiriX® v.3.9.4 using, in the 3D viewer tab, the 3D Volume Rendering mode of visualization and measured using a measurement tool. The obtained data were recorded in an Excel® sheet. Six orbital measurements were taken (orbital breadth—bilateral, orbital height—bilateral, biorbital breadth, interorbital breadth, bimaxillary breadth, and zygoorbitale breadth).<sup>1</sup> Morphological analysis was based on the orbital shape and scored as 2: hyperfeminine; -1: feminine; 0: neutral/undeterminate; +1: masculine; +2: hypermasculine.<sup>2</sup> Statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) with the statistical significance set at  $P=0.05$ . The normality of distribution was tested with the Shapiro-Wilk test. The accuracy of the discriminant functions was evaluated in the original and cross-validated samples.

**Results:** Measurements interorbital breadth, bimaxillary breadth, zygoorbitale breadth, and the left-sided orbital breadth were excluded because of the inter-observer error. For the inter-observer error in morphology, Weighted Kappa was 0.41520 (moderate agreement). There were no statistically significant differences between the right and the left side of orbital width and height. Males and females exhibited statistically significant sexual dimorphism except for the interorbital breadth ( $P=0.114$ ). The accuracy of the orbital measurements varies, with the lowest in zygoorbitale breadth (55%) and the highest for the biorbital breadth (72.5%) and left orbital breadth (73.5%). The discriminant functions of classification rated from 69% to 70.5%. Morphological traits exhibited statistically significant sexual dimorphism ( $P<0.001$ ) and a high classification rate (94.5%).

**Conclusion:** The most valuable orbital measurements for sex estimation in the Croatian population are the orbital width and height as well as the biorbital breadth. On the other hand, the interorbital breadth did not exhibit sexual dimorphism in the Croatian contemporary population. The highest value of the classification rate/accuracy for the orbital measurements was 70.5%, which is not appropriate for forensic sex estimation. The morphological traits exhibited high sexual dimorphism and high classification rate. Thus, in this example, the morphological traits are the more valuable sex indicator, rather than the metric traits.

### Reference(s):

1. Langley N.R., Jantz L.M., Ousley S.D., Jantz R.L., Milner G. Data collection procedures for forensic skeletal material 2.0. *University of Tennessee and Lincoln Memorial University*; 2016, Knoxville, TN.
2. Acsádi G., Nemeskéri J., Balás K. History of human life span and mortality. *Akadémiai Kiadó Budapest*; 1970, Budapest, EU.

### Sex Estimation, Computed Tomography, Orbital Area

## A11 A Preliminary Study of Sex Determination Using the Alveolar Ridge in Korean Adults

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**Learning Overview:** After attending this presentation, attendees will understand the sexual dimorphism existing in the alveolar ridge and how the alveolar ridge can perform as an indicator of sex in cases where other sex determinants in the cranium are insufficient.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an option to determine the sex of a skull without more conspicuous sexual indicators and enlarge the chance of collecting the information of an individual in a medicolegal investigation.

Non-metric traits of the skull, such as the ones suggested by Walker, have been a well-known method in sex determination.<sup>1</sup> The male skull tends to be larger and more robust than the female skull. The non-metric traits especially involve the occipital, temporal, frontal, and mandible as indicators of sexual dimorphism. In cases where those skeletal elements are unavailable or ambiguous, however, a researcher can opt for other parameters for sex determination.

The upper alveolar ridge consists of the maxillae's alveolar processes, and the lower alveolar ridge is a part of the mandible that contains the teeth. The average breadth of the dental arch (arrays of teeth) is larger in males than females, but few reports presented which part of the dental arch is more appropriate for sex estimation. Also, the dental arch is prone to be not intact; the teeth are often lost in burial cases because of the decaying of connecting tissue or intentionally damaged in criminal cases to hinder personal identification. Thus, researchers investigated the alveolar ridge instead of the dental arch since the alveolar ridge is a part of the maxilla and mandible, including the dental arch.

Sixty 3D models of Korean adult skulls were randomly selected from a collection of clinical computed tomography scans, with the same distribution of sex and age groups (30 females and 30 males). The five age groups were divided by decade of ages (the 20s, 30s, 40s, 50s, and 60s and above).

Using Mimics® ver. 14, landmarks were placed on the outer and inner surface of the alveolar ridges of the maxilla and mandible, between each neighboring tooth, and close to the root of the teeth. Then midpoints of outer landmarks and inner landmarks were calculated by their coordinates. Measurements were performed automatically by the program to figure out the relationships of those midpoints. Planes passing through neighboring two midpoints were created to measure the angular change of the alveolar ridge. For the lengths of the alveolar ridge in the anterior-to-posterior direction, planes passing through midpoints on each side were defined. Then the distance from the foremost midpoint to these planes were measured. Also, the breadths of the alveolar ridge between midpoints at the same level on each side were measured.

The accuracy rate of sex estimation by each measurement was calculated through a discriminant function analysis. Among maxillary measurements, breadth measurements at the premolar level showed an accuracy of over 70%. The demarcation point of Premolar1-Premolar2 (PM1PM2) breadth was 40.78mm, Premolar2-Molar1 (PM2M1) breadth was 45.83mm, and Molar1-Molar2 (M1M2) breadth was 49.92mm. The maxillary alveolar ridge's anterior-posterior length scored the highest accuracy of all maxillary measurements with an accuracy of 78.3%; the demarcation point was 48.90mm. The angle between Incisor1-Incisor2 (I1I2) right angle showed the highest accuracy among angle measurements with an accuracy of 71.7%; the demarcation point was 8.69 degrees ( $p < 0.05$ ).

For the mandibular alveolar ridge, measurements of the posterior part showed higher accuracy. Breadth and length at the level in which the mandibular rami connected to the alveolar ridge (alveolar posterior, PA) showed an accuracy of 70.0% and 71.7%, respectively. The demarcation point for the breadth at the level of PA was 83.26mm, and the length at the level of PA was 38.02mm. Also, the change of the angle at the level of Molar2-Molar3 (M2M3) showed the highest accuracy among angular measurements with an accuracy of 80.0%; the demarcation point was 56.30 degrees ( $p < 0.05$ ).

Overall, it was found that the alveolar ridge retains sexual dimorphism, which is valid for sex estimation. Especially, the part from the premolar to the molar in both alveolar ridges showed greater power of discrimination. Since the usage of 3D scanners and computed tomography is rising, applying alveolar ridge measurement to estimate the sex of a real skull would be available. However, a future study on this subject is required because there is a small number of studies dealing with the alveolar ridge for sex estimation, and extended data for various sample populations would be valuable for application in forensic anthropology.

### Reference(s):

1. Walker P.L. Sexing skulls using discriminant function analysis of visually assessed traits. *Am J Phys Anthropol.* 2008 May;136(1):39-50. doi: 10.1002/ajpa.20776. PMID: 18324631.

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### Sex Determination, Alveolar Ridge, 3D analysis

## A12 Applying Posterior Probability Thresholds to Traditional Cranial Trait Sex Estimation Methods

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**Learning Overview:** After attending this presentation, attendees will understand how Posterior Probability-Informed Threshold (PPIT) cutoffs can be used to improve the accuracy and reporting methods of sex estimations based on traditional cranial non-metric traits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting PPITs as a replacement to the dichotomous distinction (male or female estimate) based on a single sectioning point.

Currently, sex estimation methods using the traditional five cranial non-metric traits produce a final sex estimation (male or female) using the resulting score; for example, the discriminant function score, and a single sectioning point.<sup>1,2</sup> When sex estimations are gleaned from discriminant scoring alone, statistical confidence in the assigned sex is not typically assessed. The ultimate sex assignment is the same regardless of whether the individual falls close to the sectioning point or at the extremes of one of the sexes. Although some practitioners may informally report their confidence in the assessment (e.g., probable male), these confidence statements are subjective, not standardized, and not necessarily based on statistical results. The present study examines sex estimation outcomes using Garvin et al.'s non-metric cranial traits data.<sup>1</sup> The purpose of the present study is threefold: (1) to evaluate how PPITs impact accuracy, (2) assess the balance between sample inclusion and accuracy for the proposed PPIT approach, and (3) make recommendations for the use and interpretation of specific thresholds for this sex estimation method.

To assess how PPITs affect accuracy, posterior probabilities associated with the discriminant function analyses for Garvin et al.'s 2014 sample ( $n=491$ ) were used. Five PPIT cutoffs were developed ( $\geq 0.95$ ,  $\geq 0.85$ ,  $\geq 0.75$ ,  $\geq 0.65$ , and  $\geq 0.50$ ), such that a threshold of " $\geq 0.85$ " means that only individuals whose posterior probability was greater than or equal to 0.85 should be considered for sex estimation using this method. Thus, individuals with posterior probabilities less than 0.85 would result in an "indeterminate." Unsurprisingly, accuracy rates increased as the PPIT cutoff increased. Furthermore, accuracy rates were sample-dependent, with the United States White sample generally producing greater accuracy rates (0.89–0.99). Accuracy rates were also sex-dependent, with females generally displaying greater accuracies than males. Importantly, increasing the PPIT mitigated both population and sex differences in accuracies.

The study next examined how sample inclusion was impacted by the use of thresholds, given that high accuracy rates will not be beneficial in practice if the method categorizes the majority of cases as indeterminate. Sample inclusion frequency was calculated for each threshold range by dividing the number of individuals that had a posterior probability that met the threshold criteria by the total number of individuals in the available sample (those that did and did not meet the threshold criteria). Sample inclusion frequencies consistently decrease as the threshold increases. While the  $\geq 0.95$  PPIT had the greatest accuracy (0.97), it also had the lowest sample inclusion (0.49), as expected. Sample inclusion and accuracy rates were most closely balanced in the  $\geq 0.65$  and  $\geq 0.75$  PPITs.

Finally, this study examined whether the difference in accuracy rates among the thresholds differed from one another statistically to help inform a standardized approach to method interpretation of sex. A Chi-squared test and subsequent correspondence analysis indicated that significant differences in accuracies exist among the thresholds, with PPITs  $\geq 0.95$  and  $\geq 0.85$  clustering together, and away from, the remaining thresholds. PPIT  $\geq 0.50$  was not the poorest performing accuracy and clustered with the  $\geq 0.75$  accuracy rates.

The following approach to PPIT applications to cranial trait scores is recommended: cases with PPs  $< 0.75$  should be considered indeterminate, cases with PPs of 0.75–0.84 should be considered as "possible" estimated sex (e.g., "possible male"), while cases with PPs  $\geq 0.85$  should be considered as "probable" estimated sex (e.g., probable male). Utilizing PPITs can not only increase method accuracy and provide a means of reporting estimate confidence, but can also provide standardized interpretations of sex estimation results among forensic anthropologists.

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1. Garvin, Heather M., Sabrina B. Sholts, and Laurel A. Mosca. Sexual dimorphism in human cranial trait scores: effects of population, age, and body size. *American journal of physical anthropology* 154, no. 2 (2014): 259-269.
2. Walker, Phillip L. Sexing skulls using discriminant function analysis of visually assessed traits. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists* 136, no. 1 (2008): 39-50.

### Anthropology, Sex Estimation, Accuracy

## A13 Sex Estimation of Skulls and Crania From Colombia Using MorphoPASSE Program v1.0

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**Learning Overview:** After attending this presentation, attendees will have learned the utility of the MorphoPASSE program and will see how it can generate sex estimates quickly and easily based only on a handful of scored skeletal features. This presentation will also show how adjusting the input parameters of the program can increase classification accuracy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that freeware for sex estimation can be a useful tool in the estimation of the biological profile. This presentation will also impact the forensic science community by adding to research regarding the mental eminence and its unreliance for sex estimation.

MorphoPASSE v1.0 is a free program designed to use morphological scores of the skull and pelvis to estimate the sex of human skeletal remains.<sup>1</sup> This program uses random forest modeling, a flexible machine learning algorithm, which creates a series of decision trees in order to produce an average prediction. The study described here investigated the classification rates of this program when applied to skull morphology using the method described in Walker.<sup>2</sup> One hundred thirty-one skulls from the Human Skeletal Reference Collection for the Colombian Population, a modern skeletal series curated by the National Institute of Legal Medicine and Forensic Sciences in Bogotá, Colombia, were scored. The scores were input into MorphoPASSE in four different test runs to investigate which parameters of the program yielded the highest correct classification rates. These parameters are listed below.

MorphoPASSE Test 1 (MP1) included scores for skull features glabella, nuchal crest, mental eminence, supraorbital margin, and the mastoid process. For this run, all demographic criteria were set as “Unknown.” Ninety-four of the 131 cases (71.8%) were correctly classified.

MorphoPASSE Test 2 (MP2) included scores for cranial features glabella, nuchal crest, supraorbital margin, and mastoid process; however, it excluded the score for mental eminence due to issues with high inter-observer error.<sup>1</sup> For this run, all demographic criteria were set as “Unknown.” One hundred two of the 131 cases (77.9%) were correctly classified. Excluding the score for mental eminence therefore resulted in an increase of correct classifications by 6.1%.

MorphoPASSE Test 3 (MP3) used the same skull features that were scored in MP1; however, during this test, the “Ancestry” demographic was set to “Hispanic.” In this run, 105 of the 131 cases (80.2%) were correctly classified. This increased the rate of correct classifications by a total of 8.4% compared to MP1 and an increase of 2.3% compared to MP2.

MorphoPASSE Test 4 (MP4) used the same cranial features scored in MP2; however, the “Ancestry” demographic was set to “Hispanic.” This resulted in 106 of the 131 cases (80.9%) being correctly classified. This run demonstrated an increase of correct classifications by 9.1% compared to MP1; by 3% compared to MP2; and by 0.7% compared to MP3.

The MorphoPASSE program worked reliably well in all four test runs despite two main limitations. First, the program is designed to incorporate data from the skull and pelvis; however, this study only included scores for skull features. Second, the data in this study were scored using Walker instead of the modified descriptions for the MorphoPASSE program.<sup>2</sup> Even with these limitations, the correct classification rate still ranged from 71.8% to 80.9%. Finally, as demonstrated elsewhere, the results of these analyses also suggest that the use of the mental eminence may be inappropriate for sex estimation as its inclusion resulted in reduced classification rates.

### Reference(s):

1. Klales, Alexandra R. MorphoPASSE: Morphological pelvis and skull sex estimation program. *Sex Estimation of the Human Skeleton*. Academic Press, 2020. 271-278.
2. Walker, P.L. (2008). Sexing skulls using discriminant function analysis of visually assessed traits. *American Journal of Physical Anthropology*, 136(1), 39-50.

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### Sex Estimation, Morphometrics, MorphoPASSE



## A14 The Relationship of Enthesis Size to Muscle Size and Sexual Dimorphism in the Cranium and Clavicle in New Zealand and Thai Populations

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**Learning Overview:** After attending this presentation, attendees will understand the relationship between entheses (muscle attachment sites) and muscle size from eight selected upper limb and neck muscles and how enthesal size relates to human skeletal sexual dimorphism in both the cranium and clavicle in two different populations.<sup>1-3</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining how muscle size may influence the associated enthesal area and whether enthesal measurements can predict sexual dimorphism at two skeletal landmarks on the cranium (the nuchal crest and mastoid process) and one on the clavicle (the rhomboid fossa).<sup>1-3</sup> This project is also relevant to the forensic science community as it will explore how enthesal measurements may inform the development of a metric sex estimation method from these landmarks.

The overall aim of this research is to create a valid and reliable method for estimating sex from the cranium and clavicle, and this will be achieved through the analysis of soft tissue anatomy and its relationship to sexually dimorphic areas of the skeleton. Reliable sex estimation is essential to identifying missing persons and to understanding archaeological populations, among other things. In the absence of a pelvis for sex estimation, cranial and postcranial elements can be used following existing metric and morphological methods.<sup>1-4</sup> However, morphological scoring requires experience, and these methods can be impacted by the subjectivity of the observer.<sup>5-8</sup> Although expertise is important in biological profile estimation, and subjectivity can never be fully eliminated, metric methods may help improve accuracy through the use of standardized measurements and population-specific equations.<sup>4</sup>

This presentation follows on from previous research presented at the 2020 AAFS meeting, which discussed the relationship between muscle size, sex, and the three key skeletal landmarks.<sup>9</sup> The muscles included in both studies were upper trapezius and semispinalis capitis (associated with the nuchal crest); sternocleidomastoid, splenius capitis, and longissimus capitis (associated with the mastoid processes); and the clavicular head of pectoralis major, sternohyoid, and subclavius (associated with the rhomboid fossa). The research presented here examines the specific relationship between muscle enthesal area and (1) muscle size and (2) the sexual dimorphism observed at the associated skeletal landmarks, scored using established methods.<sup>1-3</sup>

European New Zealand and Thai populations were represented in a sample of 20 bequeathed cadavers from the University of Otago (ethics reference, H18/113) and Khon Kaen University (ethics reference, HE621296). The head and necks were dissected to obtain the physiological cross-sectional area (fascicle volume/length), or overall size, of each muscle of interest (eight in total). Following the dissection of each muscle, the entheses were outlined on the skeleton using a grease pencil, and 3D scans were taken of the outlined entheses using a 3D scanner tablet attachment. Scans were then uploaded to a 3D animation program through which area measurements could be taken. Accuracy of 3D scans was assessed by completing some scans with a scale bar present and validating measurements for both length and area in the computer program.

Results from previously presented research showed differences in muscle size between males and females for all muscles studied, except for upper trapezius, right longissimus capitis, and right subclavius. However, preliminary results from this study show few significant differences between sex in relation to enthesal size. Of the 27 entheses measured, including entheses both directly related to the skeletal landmarks of interest as well as those only related to the muscles studied, 7 were significantly different, with enthesal size being larger in males compared to females. Interestingly, the right longissimus capitis showed a significant difference between sexes for enthesal size but not muscle size. This may suggest that muscle attachment size may not be related to sexual dimorphism as much as is assumed in sex estimation methods.<sup>1-3</sup> This further supports findings from the previously presented data, which showed that although there were significant differences between males and females in muscle size, there was no significant relationship between skeletal landmark scores and muscle size. Understanding the relationship of different factors to sexual dimorphism in the cranium and clavicle is an important element of discussing sex estimation in forensic anthropology and will help improve development of sex estimation methods in the future.

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### Sex Estimation, Sexual Dimorphism, Forensic Anthropology

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## A15 The Utility of the Sacrum for Sex Estimation

Alexandra R. Klales, PhD\*, Washburn University, Topeka, KS 66621; Ashley B. Maxwell, PhD, Washburn University, Topeka, KS 66621

**Learning Overview:** After attending this presentation, attendees will understand the utility of the sacrum for sex estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a test of current methods and providing recommendations for use or disuse of sacral morphology and metrics for skeletal sex estimation in forensic anthropology.

As part of the pelvis, the sacrum is considered one of the best skeletal indicators of sex. Morphological features of the sacrum used for sex estimation include: overall shape, number of segments, posterior visibility of the sacroiliac joint, degree of curvature, and the relative size and proportions of sacral segment one and the alae. The sacral morphology sex differences, as summarized by Klales and references within, include “the female sacrum is considered to be shorter, wider/broad, and less anteriorly curved than the male sacrum, which, in turn, creates a larger more ovoid pelvic inlet (gynecoid). The wider first sacral segment (S1) and alae breadth have consistently been the best distinguisher of females using the sacrum. In females, the alae are generally as wide or wider than the promontory; and in males, the alae are narrower than the promontory. The increased base breadth (S1 and large alae) creates an average sacral shape in females that is more similar to an equilateral triangle with three roughly even sides, while the male sacrum more closely resembles an isosceles triangle with the two sides being longer than the base. Males are also considered more likely to have greater than five sacral segments, which can also contribute to the overall longer appearance in some individuals. The male expression includes visibility of the articular surface, while it is not visible in the female expression. Lastly, the degree of curvature is also useful for sex estimation ... [with] the greatest degree of anterior curvature in males occurs between the S2 and S4 segments.”<sup>1</sup>

Most of these features appeared early in Krogman’s trait list and have since been included as indicators of sex in popular forensic anthropology textbooks.<sup>2-6</sup> Aside from Rogers and Saunders, who ranked several of these sacral traits in the top 15 most-effective pelvic traits for sex estimation based on precision and accuracy ranks, very few studies have independently tested their utility.<sup>7</sup> The aim of this research was to examine sex differences in the size of S1, number of sacral segments, proportional alae size, and sacral shape (equilateral-short and wide versus isosceles- long and narrow) using qualitative and quantitative sacral data ( $n=1,221$ ) from the Forensic Databank of the University of Tennessee. Measurements used included: anterior length (height), anterior superior breadth (based width), and maximum transverse diameter of base (S1).

Cross-validated (cv) discriminant function classification using S1 size was 72.8% (73.5% females, 72.4% males) based on the sectioning point of 48.75mm. The male mean was 5.47mm larger ( $p<0.001$ ); 24.6% of the sample had greater than five sacral segments and 67.4% of these were male. Cross-validated discriminant function classification of the alae proportion (%) of base breadth/width was 70.8% (74.3% females, 69.0% males), with the female average being 5.36% larger than males ( $p<0.001$ ). Neither sacral base length (49.5% combined cv accuracy) nor sacral height (54.8% combined cv accuracy) as a proxy for shape could be used to accurately predict sex. Sacral shape was further approximated by comparing the calculated side length (hypotenuse) to base breadth and calculating by what percentage the base was smaller than the sides (i.e., an isosceles versus equilateral triangle). There were significant differences ( $p<0.001$ ) between the male (base 12.02% smaller than sides) and female (base 9.52% smaller) means. However, classification accuracy was low with 37.5% for females and 72.0% for males using the sectioning point of 10.77mm.

Overall, none of the morphological traits that were assessed metrically produced high levels of classification accuracy (>75.0%) between males and females. Given that metrics of observable features (size and proportions) could not differentiate the sexes, it is advised the visual assessment of these subtly different features no longer be used to predict sex. Geometric morphometric approaches have produced higher accuracy (up to 98%) and could prove to be more useful for sex estimation from the sacrum provided there is a way to translate the research into a practical application and method.<sup>8</sup>

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### Sacrum, Sex Estimation, Biological Profile

## A16 A Pilot Study of Geomorphometric Sex Estimation on the Pelvis

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**Learning Overview:** After attending this presentation, attendees will better understand the applicability of the Decker et al. method to a modern, American sample for sex estimation.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of sex estimation of the metric methods of the innominate utilized in the Decker et al. method.<sup>1</sup>

The os coxae have proven to be the most reliable skeletal elements for sex estimation. However, most forensic anthropological sex estimation methods that utilize the pelvic girdle are qualitative and highly subjective. Quantitative measurements can offer *Daubert* compliance, lower error rates, better statistical backing, and reduced bias. Decker et al. proposed a quantitative method based on 20 measurements of the articulated pelvic girdle from computed tomography scans, reporting a 100% accuracy rate and low inter-observer error, using a four-variable formula.<sup>1</sup> In this study, the applicability of the Decker et al. method on dry bone is investigated, with bone preservation and articulation taken into consideration.<sup>1</sup> Additionally, this study proposes the addition of a rarely utilized measurement with variables from Decker et al.: the distance between the superior pubic symphysis and apex of the auricular surface.<sup>1,2</sup> This measurement was chosen due to variability in preservation. When skeletal remains are recovered, it is common to recover only one innominate. For these purposes, the correlation of the proposed variable with the Decker et al. measurements, as well as the effect of its addition on accuracy and precision, is examined.<sup>1</sup> From the validation study of Decker and the implementation of the new measurement, the goal is to improve necessary, quantitative analyses while simultaneously shedding light on how and why new estimation methods can be continuously improved. It is hypothesized that the Decker et al. method will prove to be less accurate when applied to skeletal remains due to concerns regarding preservation and articulation.<sup>1</sup> The added measurement will show correlation with other measurements to create a new formula with considerations on which landmarks preserve best.

A preliminary sample size of 32 modern individuals from the Mercyhurst University Collection is utilized in this sample. Either the left os coxa, right os coxa, or the entire pelvic girdle were used for data collection. All measurements from Decker et al. were taken when possible.<sup>1</sup> The sample is from Mercyhurst University's donation program, with the majority of individuals originating from the Northeastern area of the United States. Most individuals had known demographic information associated with their donation, particularly sex. For those whose sex was unknown, the Klales qualitative method was used to estimate sex.<sup>3</sup>

Results reveal low collinearities between the variables in the Decker et al. method, suggesting that most included variables contribute relevant information to the diagnosis, and their model does not appear to suffer from overfitting<sup>1</sup>. Multivariate analyses further support the validity of the Decker et al. method, indicating that the variables in the original method capture a large proportion of size-free shape variation, with large variances explained in most variable combinations, a number of significant roots exceeding 4–5 in all multivariate combinations and methods, and size explaining a proportion of the variance only in the 30%–40% range.<sup>1</sup> However, the application to this collection sample revealed many of the required landmarks in the method are frequently missing in forensic cases, resulting in a decrease in accuracy inversely proportional to the number of preserved variables. Certain variables also displayed larger inter-observer errors, which could affect accuracy. Within this framework, the newly proposed variable demonstrated to be easily and consistently measurable, frequently preserved, and a useful addition to the variables in the original method.

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### Sex Estimation, Morphometrics, Forensic Anthropology

## A17 Establishing Postcranial Sex Estimation Criteria for Unidentified Migrants at the United States-Mexico Border

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**Learning Overview:** After attending this presentation, attendees will better understand the need for broader, population-oriented sex estimation criteria that encompass biological variation of current migrant groups perishing at the United States-Mexico border.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the utility of a multi-regional postcranial sex estimation method for the identification of Latin American migrants at the United States-Mexico border.

Sex estimation is a critical part of the biological profile that greatly assists in the identification of unknown individuals. With increasing numbers of Central Americans and Mexicans crossing the United States southern border, the need for sex estimation criteria that encompass the biological variation of unidentified migrants remains critical. The current research addresses whether univariate analyses of postcranial measurements can be used to develop accurate sex classification criteria for unidentified migrants from varied regions in Latin America. Additionally, the current research compared the multi-group univariate criteria to two current postcranial sex estimation methods developed for Mexican and Guatemalan Hispanics to assess their relative classification accuracies.<sup>1,2</sup>

Utilizing a combined sample of 417 (315 males, 102 females) known-sex Central American and Mexican individuals from the Forensic Anthropology Foundation of Guatemala, Operation Identification, the Pima County Office of the Medical Examiner, Universidad Nacional Autónoma de México, and Universidad Nacional Autónoma de Yucatán, analysis of variance was conducted on 42 postcranial measurements to generate sectioning points to serve as sex estimation criteria. In order to test classification accuracies, 18 measurements with significant F-values were applied to a test sample of 29 known-sex individuals not included in the original analyses. Classification accuracies for the test sample were above 80.00% for 5 of the 18 measurements, with scapula maximum height performing best at 94.74%, followed by humerus maximum head diameter at 91.30%, and tibia maximum diameter at the nutrient foramen at 85.71%. Classification accuracies were overall better for males than females.

To compare the classification accuracies of the current Mexican (Spradley et al.) and Guatemalan (Fowler and Hughes) Hispanic postcranial sex estimation methods, all sectioning points generated by each method were applied to the same validation sample of 29 known-sex individuals.<sup>1,2</sup> Of the nine sectioning points from the Spradley et al. method, three produced classification accuracies above 85%, with the scapula height performing best at 95.74%, followed by femur maximum head diameter at 88.46%, and humerus epicondylar breadth at 88%.<sup>1</sup> The humerus maximum head diameter, scapula maximum height, and tibia maximum diameter at the nutrient foramen performed well for both the present univariate analysis and in the Spradley et al. method.<sup>1</sup> Of these three corresponding top-performing measurements, sectioning points for humerus maximum head and scapula maximum height were a millimeter smaller in the new criteria than those generated by the Spradley et al. method, suggesting their applicability to individuals of smaller body sizes.<sup>1</sup>

The Fowler and Hughes method produced one top-performing sectioning point, with the glenoid cavity height performing at 90% classification accuracy.<sup>2</sup> Of the nine measurements in the Fowler and Hughes criteria, eight sectioning points performed above 80% in males, while two performed above 90% in females, suggesting that classification accuracies decrease when sectioning points are applied to larger body sizes.<sup>2</sup> The sectioning point for the femur maximum head diameter for the Fowler and Hughes method was also identical to the one generated by the new criteria, performing at 84.62%.<sup>2</sup>

Combining the best-performing sectioning points from the current Guatemalan and Mexican Hispanic methods with the newly developed multi-group criteria may serve as the most accurate univariate approach for postcranial sex estimation for unidentified migrants. Future studies will apply these univariate criteria to additional groups of known-sex individuals, as well as develop multivariate criteria as more population data become available. Results will be discussed within the framework of population structure.

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### Sex Estimation, Unidentified Migrants, Biological Variation

## A18 An Evaluation of Patellar Measurements for Estimating Sex in African Americans

Eric Fraumenhofer\*, Buffalo, NY 14223

**Learning Overview:** After attending this presentation, attendees will better understand the accuracy of discriminant functions derived from patellar measurements for estimating the sex of African Americans as well as better understand the implications of these functions on the biological profile while attempting to identify unknown individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive evaluation of patellar measurements for estimating the sex of African Americans and will identify the most accurate methods for forensic anthropologists to use for estimating sex from patellar measurements.

Sex estimation is an important aspect of the biological profile used to identify unknown human skeletal remains. Determining sex can exclude nearly half of individuals in missing person databases, and it can help with estimating other aspects of the biological profile such as ancestry and stature. Sex is most accurately estimated using a complete skeleton; however, forensic anthropologists do not often work with complete skeletons, but rather partial skeletons or even fragments of skeletal remains. In the absence of the skull and pelvis, forensic anthropologists must rely on methods that do not use either of these elements for estimating sex. It is therefore useful to develop methods that can estimate sex based on skeletal elements other than the skull or pelvis.

Generally, there are two types of methods for estimating sex: non-metric methods that rely on observation of the skeleton, and metric methods that rely on measurements of the skeleton. One of the most commonly used metric methods for sex estimation is Discriminant Function Analysis (DFA), which has been applied to many bones for estimating sex, including the vertebrae, sternum, upper limb bones, pelvis, and lower limb bones, among others. One of the major limitations of DFA is that it is population specific, meaning functions derived from one population may not be accurate when applied to another population. Another limitation of DFA is that although many methods have been derived from various bones of various populations, not much research has examined the reliability of these methods.

Therefore, the goal of this current research was to evaluate the reliability of a recent method developed by Peckmann and Fisher for estimating the sex of African Americans using patellar measurements.<sup>1</sup> Peckmann and Fisher derived discriminant functions from the patellar measurements of 200 African American individuals (100 females and 100 males) from the Robert J. Terry Anatomical Skeleton Collection at the Smithsonian Institute's National Museum of Natural History.<sup>1</sup> For the current study, 200 African American individuals (100 females and 100 males) from the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History were studied. Following Peckmann and Fisher, six measurements were taken of each individual's left patella, including the patella's maximum height, maximum breadth, maximum thickness, height of articular facet, lateral articular facet breadth, and medial articular facet breadth.<sup>1</sup> These measurements were then entered into the discriminant functions developed by Peckmann and Fisher and the overall accuracy rates of these functions for sex classification were recorded and compared to those obtained by Peckmann and Fisher.<sup>1</sup> The overall accuracy of sex classification obtained from the current study ranged from 75.0% to 83.5% for the direct method and 75.0% to 81.0% for the stepwise method. These values are compared with accuracy rates of 80.0% to 85.0% for the direct method and 80.0% to 84.5% for the stepwise method as obtained by Peckmann and Fisher.<sup>1</sup> In the current study, the most accurate functions contained all the variables, whereas the least accurate functions contained only two variables, the maximum height and the height of the articular facet, consistent with the findings of Peckmann and Fisher.<sup>1</sup>

These findings suggest the discriminant functions developed by Peckmann and Fisher from patellar measurements can be used with caution for estimating sex in African Americans.<sup>1</sup> However, the patella should only be used for sex estimation when other bones that are more accurate for estimating sex, such as the bones of the skull or pelvis, are unavailable.

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### Sex Estimation, Patella, African American

## A19 The Iliac Crest as a Skeletal Indicator of Puberty and Guide to Subadult Sex Estimation

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**Learning Overview:** After attending this presentation, attendees will understand how the iliac crest can serve as a skeletal indicator of puberty and can aid in determining when morphological sex traits of the pelvis can be applied to immature remains with accuracy rates similar to those observed in adults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an approach to subadult sex estimation that is independent of age at death.

Morphological traits of the pelvis, including the ventral arc, subpubic contour, and medial aspect of the ischiopubic ramus, have long been known to provide the most accurate sex estimates from the human skeleton. Currently, these traits are considered “adult” traits, and consequently are typically only applied in individuals estimated to be around 18 years of age and older. However, since these traits are associated with pubertal changes to the female pelvis, they should be applicable in individuals prior to adulthood. Recent research has shown that these pelvic traits attain accuracies as high as 86.4% by 13 years of age, and accuracy rates comparable to those observed in adults (> 90%) are attained by 15 years of age.<sup>1</sup> Despite these encouraging results, the application of these traits in subadults requires age to be estimated, which could result in compounded errors if the age estimate is incorrect. Further, it is difficult to incorporate an age range into sex estimation methods.

To avoid the need for age to be known when estimating sex from the subadult pelvis, the current study uses a skeletal maturity indicator associated with puberty, the iliac crest, to determine when during ossification and fusion sex can be accurately estimated. Ossification of the iliac crest is associated with menarche in females.<sup>2,3</sup> As menarche can occur at various ages, the use of this indicator removes the need to accurately estimate age and/or the progression of puberty. Therefore, it is predicted that the absence, ossification, and fusion of this epiphysis could indicate whether or not sex estimation should be attempted in immature individuals. To determine stage of iliac crest development and sex estimation accuracy, a total of 232 individuals (m = 120, f = 112) aged between 8.29 and 20.92 years were assessed using Multi-Slice Computed Tomography (MSCT) postmortem scans from the Subadult Virtual Anthropology Database. The sample was divided into four groups dependent on stage of iliac crest development, which was evaluated as follows: Stage 9 = iliac crest is absent, Stage 0 = iliac crest is present but unfused, Stage 1 = iliac crest is partially fused, and Stage 2 = iliac crest is completely fused. Sex estimation accuracies were calculated for each group and each sex using the Klales et al. method.<sup>4</sup>

Sex estimation accuracies for subadult males ranged between 93.3% and 100% for all iliac crest development stages. This finding was expected, since male pelves generally exhibit male morphology throughout life, with changes in morphology being more associated with females resulting from downstream effects of puberty. Female accuracy for Stage 9 (8.29–13.47 years) was poor, with only a 12% accuracy rate being achieved. Accuracy increased dramatically for females in Stage 0 (10.80–19.93 years), with 81.5% being correctly classified. Female accuracies for Stages 1 (14.48–20.08 years) and 2 (13.17–20.92 years) were 100%. The combined sex accuracy for individuals exhibiting partial or complete fusion of the iliac crest (Stages 1 and 2) was 98.3% (m = 96.7%, f = 100%).

The results of this research illustrate that the iliac crest can serve as a skeletal indicator of puberty, which can serve as a guide for practitioners in deciding whether sex estimation is possible in immature skeletal remains. Specifically, sex estimation should not be attempted in subadults prior to iliac crest ossification. However, sex estimation can be confidently estimated when fusion of the iliac crest has commenced. This approach is convenient since both the maturation and sex indicators are present on the same skeletal element, as well as accurate, since it does not rely on a correct age-at-death estimate.

*This research was funded in part by the National Institute of Justice (NIJ) (2015-DN-BX-K409). The opinions, findings, conclusions, and recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the NIJ.*

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### Sex Estimation, Juvenile, Puberty

## A20 Age Estimation in a South African Sample of 224 Living Subjects Using the Cameriere (Bo/Ca) and Tanner–Whitehouse 2 (TW2) Methods

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**Learning Overview:** The goal of this presentation is to compare the accuracy of skeletal age versus chronological age assessments using the Bo/Ca and TW2 methods in a South African sample of 224 individuals, aged between 6 and 16 years old, focusing on ethnicity and gender.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by verifying the applicability of a new method (Bo/Ca) for forensic age estimation in a specific population.

Hand and wrist X-rays represent the most common and objective method for assessing skeletal age.<sup>1</sup> Several qualitative and quantitative bone-specific methods have been developed, such as the Greulich and Payle atlas and the Tanner and Whitehouse method, refined and updated as TW2 and TW3.<sup>2-5</sup> A more recent quantitative method was designed by Cameriere et al. in 2006 (Bo/Ca method) using a computer-aided software for the measurements of hand and wrist bones on X-rays.<sup>6</sup> The applicability of this new method has been little investigated and only limited data are available on the accuracy of the Bo/Ca method for specific populations.

The aim of the research was to compare the accuracy of Skeletal Age (SA) versus Chronological Age (CA) assessments using the Bo/Ca and TW2 methods in a large sample of children and adolescents from South Africa.

A retrospective study was performed using a digital database consisting of 224 hand-wrist radiographs. The sample consisted of 110 Black (55 females, 55 males) and 114 White European ethnicity subadults living in South Africa (56 females, 58 males). The CA ranged between 6 and 16 years (yr) with a mean age of 12.3yrs. The SA was estimated by two observers trained in skeletal age assessment, separately and independently, using the Bo/Ca method and the Radius-Ulna-Short bones (RUS), ossa Carpi compartment (CARP) and TW2-20 score methods. The differences between SA and CA estimates were used as indicators of the performance of the applied methods. Accuracy, sensitivity, and specificity of Bo/Ca and TW2 methods were calculated based on the age limit of 13 years. The inter-observer reliability was calculated by means of the Intra-Class Correlation (ICC).

Both methods showed a small discrepancy between SA and CA estimates, since the median values fall mostly within two months either side. Bo/Ca and Tw2 correctly classified both White (-0.08 and 0.18yr, respectively) and Black individuals (-0.07 and -0.20yr, respectively) and both males (-0.19 and 0.19yr, respectively) and females (-0.03 and -0.21yr, respectively). BO/Ca and TW2 methods showed a greater inaccuracy in subadults aged 13yrs or older compared with children younger than 13yrs old. SA estimates performed by Bo/Ca seem to be less influenced by ethnicity or gender than the TW2 method. Finally, the inter-observer reliability was found to be very small to a near-complete agreement, as demonstrated by the ICC (0.998; 95%CI 0.998–0.999).

According to this study, both Bo/Ca and TW2 radiological methods can be reliable for forensic age estimation of living individuals in South Africa. However, limitations dealing with normal variation in skeletal maturation due to nutritional and gender variables must be taken into account as well as the differences in the onset of pubertal development between ethnic groups. According to a holistic and multidisciplinary approach, in any forensic age estimation process it would be appropriate to consider the information on skeletal development provided by Bo/Ca and TW2 methods.

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### Age Estimation, Skeletal Age Assessment, Anthropology Population Data

## A21 A Computed Tomographic Evaluation of the Vertebrochondral Joint for Age Estimation

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**Learning Overview:** The goal of this presentation is to provide the scientific community with a new method of age estimation that involves computed tomographic visualization of the vertebral ends of ribs for assessing the degree of ossification of the heads of ribs. Linear regression models developed using these scores can help forensic anthropologists in estimating age.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shedding light on a novel method of age estimation that scores the degree of ossification of heads of the ribs. Linear regression models developed using these scores can help forensic anthropologists in estimating age. The scores themselves can be used to ascertain whether an individual has attained the medicolegally significant ages of 16 and 18 years.

Age estimation is a crucial part of the identification process. It has significant medicolegal implications in the living, in both civil and criminal law. Age estimation is routinely conducted in cases of individuals participating in sports events, applying for jobs, pensions, etc. It is done in the identification of unaccompanied minors, asylum seekers, and refugees lacking proper documentation indicative of their age. It is vital in the process of assigning criminal responsibility, and is also done in victims of child labor, prostitution and pornography. The most common methods of age estimation are the ones that assess the degree of ossification/fusion of certain indicators of skeletal maturity.

While most of these indicators have been researched thoroughly, one of the less explored anatomical regions whose degree of ossification may have the potential to be used in age estimation practices is the heads of the ribs. The ossification centers pertaining to the heads of the ribs appear by the age of puberty and attain complete maturity by 22–25 years of age. Only a few studies globally have investigated the time of ossification of heads of ribs, and all of these have been conducted on skeletal remains. However, the advent of Computed Tomography (CT) has allowed scientists to visualize previously inaccessible indicators of skeletal maturity in the living. CT can be used to visualize the heads of the ribs, which are attached to the vertebrae by means of an articular facet. The present study visualized the heads of all the ribs of 148 participants (74 males and 74 females) aged 10 to 25 years using CT and scored their ossification status using a modification of the system used by Rios and Cardoso.<sup>1</sup> It was observed that the mean age at any stage of ossification for both the sexes was higher for the anterior and posterior ribs as compared to the middle ribs, indicating that the middle ribs ossify later than the anterior and posterior ones. No bilateral differences or sexual dimorphism was observed ( $p > 0.05$ ). A statistically significant positive correlation ( $p < 0.001$ ) was observed between the ossification scores of each ribs' heads and the chronological age of all the participants. Regression models to estimate age were generated using the rib ossification scores. It was observed that depending on the degree of ossification of the heads of the ribs, it can be ascertained whether an individual has attained the medicolegally significant ages of 16 and 18 years.

This study is the first of its kind, as no other study which uses CT to observe degree of ossification of the heads of ribs for age estimation has ever been performed. The results of this study will provide forensic anthropologists and medicolegal experts from across the globe with a novel method of age estimation. The regression models developed in this study can be used to estimate age using rib head ossification in the Southeastern Asian population.

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### Age Estimation, Head of Ribs, Computed Tomography



## A22 Age Estimation Using a Radiation-Free Medical Method: An Analysis of Carpal Bones by Magnetic Resonance Imaging (MRI)

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**Learning Overview:** The goal of this presentation is to verify the applicability of a new radiation-free medical method for age estimation in a sample of 57 individuals between 12 and 20 years of age.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new method for age estimation based on hand and wrist bone analyses by MRI.

In age estimation of living individuals, there is growing interest in using MRI to avoid exposure to ionizing radiation. Moreover, MRI compared to radiography has more details because it is able to define the individual ossification nuclei and the cartilage that surrounds single bones. Based on Bo/Ca method by Cameriere et al., the purpose of this study is to investigate the possibility of using MRI on carpal bones for age estimation.<sup>1</sup>

A retrospective study was performed analyzing 57 MRI scans of Italian subjects aged between 12 and 20 years, without growth diseases, endocrine disorders, or osteodystrophy. MRIs have been extrapolated and subsequently processed using a computer-aided drafting program (ImageJ). For each carpal bone, the ratio (NOSG) between the area occupied by the Nucleus of Ossification (NO) and the Surface of Growth (SG) was calculated; the latter was derived by adding the NO to the area of cartilage-bone interface. This procedure was carried out using a polygonal selection tool, capable of delimiting an area through a series of linear segments.

For age estimation, this study obtained the following multiple linear regression formula:

$$\text{Age} = -20.1743 + 0.2644 \cdot g + 3.0206 \cdot Td + 4.1685 \cdot Tm + 3.9135 \cdot S + 13.3617 \cdot P + 20.9222 \cdot C$$

where:

g = 1 for male and 0 for female

Td is NOSG<sub>Trapezoid</sub>

Tm is NOSG<sub>Trapezium</sub>

S is NOSG<sub>Scaphoid</sub>

P is NOSG<sub>Pisiform</sub>

C is NOSG<sub>Capitate</sub>

The median of the residuals (observed age minus predicted age) was -0.025 years, with an Interquartile Range (IQR) of 0.19 years. The results demonstrated that the best model, with the lowest Root Mean Square Error (RMSE) and the highest coefficient of determination ( $R^2$ ), was obtained with six predictors ( $N_{\text{vmax}}=6$ ): Gender, and the NOSG of the trapezoid, trapezium, scaphoid, pisiform, and capitate.

According to this study, thanks to the improved resolution of MRI on carpal bones, the new method could allow one to define the age of minors with an extremely low margin of error and to obtain more accurate results for subjects over 18 years of age. In fact, the sample contained nine individuals over the age of 19, which is a not negligible number given the age range of the sample population (i.e., 12–20 years). However, to verify the applicability of the method in the forensic field, studies on larger samples and populations of different ethnic origin will be needed in the future.

Furthermore, a use of the new method on skeletal remains is also envisaged through the realization of specific regression models for each of the five carpal bones considered in the formula, for which a coefficient of determination  $R^2 > 0.9$  was found. This could be useful for identification/age estimation purposes in dead bodies or in the event of the discovery of portions of the hand (as a result, for example, of mass disaster or fragmentation due to the action of macrofauna).

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### Age Estimation, Magnetic Resonance Imaging, Carpal Bones

## A23 A Computed Tomographic Analysis of Medial Clavicular Epiphyseal Fusion for Age Estimation in an Indian Population

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**Learning Overview:** The goal of this presentation is to showcase the results of the first Computed Tomography (CT)-based study on medial clavicular epiphyseal fusion in an Indian population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community across the globe and especially in the Indian subcontinent by detailing how to estimate age in the living using medial clavicular epiphysis.

Forensic age estimation is one of the “Big 4” of identification and is routinely conducted in identification of both the living and dead. While the conventional radiographic visualization of epiphyseal fusion of long bones for the purpose of age estimation has been researched exhaustively over the 20<sup>th</sup> century, recently forensic anthropologists and scientists have shifted their focus to the use of medial clavicular epiphyseal fusion in age estimation. Not only can the medial clavicular epiphyseal fusion be used to estimate age in young adults, but it can also be used to determine whether a person has attained the medicolegally significant ages of 16 and 18 years.

The present study aimed at visualizing the status of medial clavicular epiphyseal fusion using CT in an Indian population, scoring the stages of fusion using the methods by Schmeling et al. and Kellinghaus et al., generating regression models using these scores, applying these regression models on a test set to study the variance between chronological age and estimated age of the test set participants, and assessing whether an individual has attained the medicolegally significant ages of 16 and 18 years old. Medial clavicular epiphyseal fusion of 350 participants (147 females and 203 males) aged 10.01–35.47 years was studied to generate regression models. Statistically significant correlation ( $p < 0.005$ ) was observed between the degree of fusion and the chronological age of the participants ( $\rho = 0.918$  in females, and  $\rho = 0.905$  in males). The regression models generated using the sample set of 350 participants when applied on the test set of 50 participants (25 females, 25 males) showed a mean absolute error of 1.50 for females, 1.14 for males, and 1.32 for the total test set. It was also observed in the present study that all the individuals of either sex, with the incidence of stage 3a or above degree of medial clavicular epiphyseal fusion, were older than 18 years of age. Similarly, presence of stage 1 of the clavicular epiphyseal fusion indicated the individual to be less than 18 years old. The cut off for an individual to be at least 16 years of age was observed to be stage 2b for males and stage 2c in females. For males, individuals with stage 2b and above are definitely at least 16 years old or more, while in females, observation of stage 2c and above in an individual indicates that they are definitely at least 16 years old or more.

The present study is the first CT-based investigation of the medial clavicular epiphyseal fusion’s utility in age estimation in the Indian subcontinent. The results of this study will assist the forensic anthropologists and medicolegal professionals of the Indian subcontinent and the rest of the world to accurately estimate age using the medial clavicular epiphyseal fusion in their respective populations.

### Age Estimation, Medial Clavicular Epiphysis, Computed Tomography

## A24 Estimating Age From 2D and 3D Imaging of Skeletal Remains: An Assessment of Reliability Using the Medial Clavicle

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**Learning Overview:** After attending this presentation, attendees will be familiar with the reliable use of photographs and 3D scans for assessing medial clavicle fusion, including the effects of experience level and scoring method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating: (1) that observers generally produce more reliable scores using photographs compared to 3D scans; (2) that osteological experience improved reliability of assessment for both viewing modalities; in addition to (3) supporting previous research that the application of a three-phase method of scoring the medial clavicle, rather than five-phase method, is less subjective and thus more reliable.

Research regarding the utility of digital imagery in conducting preliminary remote forensic analyses, or analyses of remains occurring outside of the laboratory, is necessary for the progression of forensic anthropology as new technologies arise. Further, examination of the use of high-resolution imagery for subsequent study of remains is integral for understanding the validity and biases of using photography and 3D scanning for forensic research. For these reasons, it is necessary to determine if photograph and 3D scan modalities can be reliably used for the macroscopic assessment of skeletal remains for age estimation when the physical remains are not available. This study evaluated whether or not an assessment of the developmental stage of the medial clavicle can be replicated consistently among macroscopic analysis of the physical skeletal element and a photograph or 3D scan of the same element.

This research was conducted at the Defense POW/MIA Accounting Agency (DPAA) Laboratory at Offutt Air Force Base, NE, utilizing remains of service members killed on the USS *Oklahoma* during the Japanese attack at Pearl Harbor, HI. The relative reliability of scores taken from each digital modality was assessed. The age estimation method used was the McKern and Stewart five-phase scoring method.<sup>1</sup> Scores were also collapsed into the Langley-Shirley and Jantz three-phase scoring method to assess the effect of different scoring methods on reliability.<sup>2</sup> It is important to emphasize that this study did not assess the accuracy of the aforementioned age estimation methods, but instead addressed the replicability of scoring an element using digital imagery in place of the physical element. The experience level of participants was also taken into account by determining if the ability to reliably assess developmental phase from each modality was affected by the extent of observer experience. Participant groups comprised those with extensive osteological experience ( $n=3$ ) and no osteological experience ( $n=3$ ). Intra-observer error for each modality and method was assessed using intra-class correlation. The absolute difference of scores for each modality compared to the physical remains was calculated for the pooled observer groups and for each observer group using each scoring method; differences greater than one phase were also assessed. Weighted kappa was used to estimate observer agreement for each phase compared to the physical remains.

Intra-observer reliability was excellent when using photographs and good when using 3D scans for both scoring methods. Observations from photographs were more reliable than 3D scans; however, experienced participants produced more reliable scores using photographs and inexperienced participants produced more reliable scores using 3D scans. Differences in reliability between observer groups show that osteological experience is necessary for both modalities. In addition, the three-phase scoring method was found to produce more reliable scores than the five-phase scoring method, indicating that the three-phase method is less subjective and thus more reliable.

These findings support the use of both photograph and 3D scan modalities for assessing epiphyseal fusion of human remains for observers with osteological experience. The remote assessment of remains can thus be reliably applied to preliminary medicolegal casework, which may help quicken the identification process. Further, this research also supports the use of digital photograph and 3D archives of remains for future study and biological profile development, thus expanding the availability of human remains for research and making research collections more accessible. Finally, this research also supports the use of digital inter-lab proficiency testing, which could lead to training standardization among laboratories.

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### Medial Clavicle, Utility, Digital Modality

## A25 The McKern-Stewart Method as a Technique for Analyzing Age-Related Pubic Symphyseal Changes: A Systematic Review and Meta-Analysis

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**Learning Overview:** The goal of this presentation is to establish the applicability of the McKern-Stewart method for age estimation by analyzing findings corresponding to mean age for the onset and progression of age-related morphological changes obtained across various studies and, also against the original findings of McKern and Stewart.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing the applicability of the aforementioned method for the purpose of age estimation. In the current scenario where a multitude of age estimation methods are explored, it becomes imperative to ascertain the accuracy and relevancy of these different research methods. The findings of component- and scoring-based analysis, in consortium with the observed uniformity in the distribution of mean ages for each score and each component across populations, points toward the reliability of this method in investigations, inclusive of and not limited to cases of forensic significance.

The McKern-Stewart method, devised in 1957, has over the years gradually evolved into one of the prominently used methods for aging skeletal remains. With age estimation studies employing the os pubis constituting a fair share of contemporary research, the McKern-Stewart method continues to be explored for analyzing age-related morphological changes incurred by the pubic symphysis. The method entails breaking down the observed changes into three distinct components, followed by assigning a specific score to the remains based on the observed morphological changes within each component. Subsequently, an age range based on the cumulative score obtained is established. The present systematic review was piloted with the aim of establishing the applicability of the McKern-Stewart method for age estimation by comparing the results obtained across various studies and also against the original findings of McKern and Stewart. The review was targeted toward isolating original research focusing on the use of the McKern-Stewart method for aging skeletal remains via systematic and standardized procedures. Studies pertaining to the use of the aforementioned method for skeletal age estimation were retrieved by appropriately keying in a combination of Medical Subject Headings (MeSH) terms, free terms, and relevant Boolean operators from four different databases—PubMed®, Cochrane Central Register of Controlled Trials (CENTRAL), Google® Scholar, and ScienceDirect®. The articles retrieved were subjected to a preliminary elimination based on the inclusion and exclusion criteria laid down, following which the risk of bias was assessed and quality of evidence was established. Once the final tally of relevant articles was obtained, data specific to mean age pertaining to individualistic and cumulative scores were extracted and subjected to meta-analysis. The Kruskal-Wallis test, along with corresponding boxplots, were used to compare findings indicative of age of the remains. Mean ages across these studies, including the data obtained from McKern-Stewart's original work, yielded a non-significant difference for each of the cumulative scores from 0–15, suggesting that there is a certain uniformity in the occurrence and prevalence of age-related pubic symphyseal changes. Furthermore, component-wise analysis also points toward a certain concordance in the distribution of mean ages corresponding to scores 0–5 individually, for each component, across different populations.

Based on the results of meta-analysis, it can be concluded that the McKern-Stewart method exhibits evidence in favor of its applicability for the purpose of age estimation. The component- and scoring-based analysis, in consortium with the observed uniformity in the distribution of mean ages for each score and each component, across populations, points toward the reliability of the method in investigations inclusive of and not limited to cases of forensic significance.

### Age Estimation, Applicability, McKern-Stewart Method

## A26 Skeletal Age Estimation From Pubic Symphysis: A Systematic Review of the Suchey-Brooks Method

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**Learning Overview:** The goal of this presentation is to elucidate the accuracy and reliability of the Suchey-Brooks method, employed for pubic symphyseal age estimation, via the analysis of Computed Tomography (CT) scans of living individuals as well as skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing to the forefront the use of radiographic modalities in age investigation studies as an alternative to the time-consuming and potentially destructive process of maceration.

Forensic age estimation is a multifaceted investigation tool, with applications in cases dealing with deceased as well as living individuals and also incorporating civil as well as criminal investigations.<sup>1-3</sup> Disparaging the bygone assumption that age estimation based on morphological changes occurring in the bones of an individual can only be analyzed postmortem, the inception of radiographic techniques such as CT has rendered scrutiny of said changes in living individuals possible. CT helps overcome the destructive and time-consuming process of maceration and helps in the analysis of these age-related morphological changes occurring throughout the skeletal framework. Over the past century, a myriad of methods have been devised for age estimation from skeletal remains. One such method, proposed by Suchey and Brooks in 1990, grouped the observed changes occurring within the pubic symphysis into six distinct phases, each defined by a corresponding age range.<sup>4</sup> The present study was piloted to determine the accuracy of the Suchey-Brooks method in computed tomographic age estimation by analyzing morphological changes occurring in the pubic symphysis of skeletal remains as well as living individuals. Original articles pertaining to the use of the Suchey-Brooks method for CT-based age estimation were extracted from four different databases—PubMed®, Cochrane Central Register of Controlled Trials (CENTRAL), Google® Scholar, and ScienceDirect®—using an adequately operative search strategy. Research papers that appeared to answer the focused question were further selected for data analysis. After assessing the risk of bias of the relevant observational studies, the data retrieved were subjected to meta-analysis.<sup>5</sup> Pooled, phase-wise, and subgroup analysis was performed to establish and verify the obtained results indicative of accuracy of the aforementioned method. Pooled analysis of correctly/accurately aged individuals/remains using the random and fixed effect models yielded a prediction percentage of 78% and 86%, respectively. Higher percentages of 92% and 82% were obtained with the fixed and random effect models respectively, for phase-wise analysis. Prediction percentages with subgroup analysis conclusively showed that morphological changes associated with Phases I, IV, V, and VI act as excellent indicators of age, whereas Phases II and III yield a comparatively lower accuracy. Results of meta-analysis indicate that the Suchey-Brooks method is a reliable method for age estimation studies. This systematic review aids in ascertaining the applicability of the Suchey-Brooks method via a thorough analysis of data related to accuracy from studies carried out across different populations. Additionally, it also verifies the fact that CT examination of the same furnishes satisfactory results, thus advocating its relevance in investigations pertaining to the field of forensic anthropology.

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### Age Estimation, Computed Tomography, Suchey-Brooks

## A27 The Age-Informative Value of the Pubic Symphysis Compared to Other Skeletal Traits in a Chilean Sample Using Transition Analysis

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**Learning Overview:** After attending this presentation, attendees will have a more nuanced understanding of the informative value of pubic symphysis trait variants for adult age-at-death estimation and a visual appreciation of the potential of combining different traits in the skeleton using transition analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new perspective on historical assumptions regarding the age-informative value of the pubic symphysis, a better understanding of the age-related changes of the evaluated trait variants, and their potential combination. This presentation also opens questions regarding the evaluation of skeletal traits as components or phases. More than any single result, this work offers additional evidence that new approaches for the identification and evaluation of age-informative traits beyond the traditional areas is needed.

This work proposes that the age-related informative value of the pubic symphysis—historically considered one of the most informative and reliable age indicators in the skeleton—has been overvalued compared to information found elsewhere in the skeleton. This research supports the idea that morphological changes of the pubic symphysis are insufficient to provide enough information to estimate age at death for middle-aged and older adults.

Pubic symphysis age-estimation methods that evaluate phases (combined traits) and report descriptive statistics are among the most used by forensic anthropologists despite documented biological and statistical issues. To evaluate the informative value of the pubic symphysis, its phases and traits were compared to the newly proposed Transition Analysis 3 (TA3) traits distributed throughout the skeleton.<sup>1</sup> The statistical approach of transition analysis has the potential to combine the information from multiple traits without forcing them to follow a pattern such as pre-determined phases, allowing each to inform different parts of the lifespan.<sup>2</sup> In this study, the collective age-informative value of the traits was assessed by visualizing the distribution of their transition curves throughout the adult lifespan.

Suchey-Brooks and Hartnett pubic symphysis phases and TA3 traits, including a small number from the pubic symphysis, were evaluated in a sample of 309 individuals from the Santiago Subactual Osteology Collection, a Chilean collection composed of unclaimed individuals who died during the second half of the 20<sup>th</sup> century.<sup>3,4</sup> Transition curves were generated for each documented transition with a pooled male and female sample and overlapped to facilitate visualization.

Considering only data from the pubic symphysis, both the TA3 traits and phases from the Suchey-Brooks and Hartnett methods were found to be only informative for a limited length of the lifespan. The highest age-informative value was concentrated in the first half of the lifespan as originally recognized by Todd and sparsely informative for the second half. Hartnett's phase VII predictably informed older ages; however, separated into components, its traits were not as informative as when grouped. TA3 traits from other areas of the skeleton inform of a larger portion of the lifespan than the pubic symphysis and do so more robustly. This improved age-informative value is due not only to TA3 traits outnumbering the evaluated pubic symphysis traits, but mainly because the traits from other areas of the skeleton provide information for different ages at death that are more widely distributed along the lifespan. Although still valuable for age estimation, the pubic symphysis does not seem to provide enough age-related information in adulthood to reduce the infamously large estimated age ranges of phase methods.

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### Age-at-Death, Pubic Symphysis, Transition Analysis

## A28 Testing the Traits of TA3: Setting a Baseline for Method Development and Performance

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**Learning Overview:** The goal of this presentation is to compare age estimates produced using the Transition Analysis (TA) method and the newly developed Transition Analysis 3 (TA3) traits for a sample of modern individuals.<sup>1,2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing baseline accuracy and precision data for estimates produced using TA3 traits in a similar statistical framework as the original TA method. These data can be used to evaluate the efficacy of alternative analytical and statistical procedures for generating age estimates from the TA3 reference sample, including sex- and population-specific approaches.

Accurate, unbiased, and reasonably precise age estimations for the entire adult lifespan are necessary for both forensic and paleodemographic applications. TA produces individualized age estimates from components of the pubic symphyses, iliac auricular surfaces, and cranial sutures. Despite using a more sophisticated statistical approach than other commonly used methods, TA performs poorly for individuals between 45–75 years of age. Although trait correlations and high inter-observer error for some features may contribute, this error is most strongly attributable to insufficient age-related change in the indicators in that portion of the lifespan.

To address this issue, a National Institute of Justice (NIJ) award was granted to an international team to identify new age-informative traits, characterize those features in modern populations, and investigate analytical approaches. The TA3 reference sample currently contains data from 1,694 documented individuals from four modern skeletal collections: William M. Bass Donated Collection ( $n=437$ ), Pretoria Bone Collection ( $n=484$ ), Chiang Mai University Skeletal Collection ( $n=418$ ), and Bocage Museum Collection ( $n=355$ ). Although initial efforts focused on directly integrating new features into a version of the existing TA framework, the number of features identified and the complexities of their correlations spurred the investigation of a number of alternative approaches.

In this study, age estimates for individuals from the University of Athens Human Skeletal Reference Collection ( $N=199$ ) were independently generated using TA and TA3 traits. Using TA software ADBOU 2.1.046, a maximum likelihood point age and 95% prediction interval was estimated for each individual based on the combined reference sample (all males and females from all samples) and a uniform prior distribution.

Of the 80 features in the TA3 Trait Manual (Public Distribution Ver. 1.0), ten features are no longer under consideration by the NIJ team and two features had definition changes between the collection of these test data and the release of the reference dataset. Probabilities for 68 traits generated from logistic models fitted to the reference data were combined to form a single log-likelihood function of age, from which a maximum likelihood point estimate and a 95% prediction interval were calculated. For non-binary traits, variants were converted to dichotomous pairs and the most age-informative transition was included in this analysis. To facilitate future comparisons with the TA3 software currently in beta testing, only data from the right side of the skeleton were used.

TA produced accurate estimates (known age fell into the estimated 95% prediction interval) for 73.3% of the individuals with an average precision (age interval length) of 34.2 years. Using the 68 TA3 traits in the simplified transition analysis procedure, the accuracy dropped marginally to 70.8%, but the precision increased to 17.2 years. In other words, accuracy remained essentially the same, while the width of the age interval was reduced by nearly 50%. This significant increase in precision without a correspondingly large reduction in accuracy is particularly surprising given that the TA program includes a statistical correction for correlated features while the procedure tested here does not. Additionally, while TA overestimated age for individuals in the first half of the lifespan, and increasingly underestimated age thereafter, the TA3 estimates exhibited no systematic age-estimation bias and were collectively indistinguishable from the identity line.

By using data calculated from the pooled reference sample (non-sex- and population-specific probabilities), a uniform prior distribution, and no correction for correlated features, this work provides a baseline for the minimum performance that can be expected using the TA3 traits, even on individuals from populations not directly represented in the reference data. Moving forward, investigating a wide array of approaches for generating estimates from the TA3 dataset will provide many avenues toward a promising future for adult age estimation.

*This research was funded in part by the National Institute of Justice (NIJ) (2014-DN-BX-K007). The opinions, findings, conclusions, and recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the NIJ.*

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### Age Estimation, Transition Analysis, TA3

## A29 Aging From Cranial Suture Closure

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**Learning Overview:** After attending this presentation, attendees will better understand a newly developed age estimation model in applying cranial suture closure for skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new approach for estimating age at death using cranial sutures, especially for Thai, Southeast Asian, and Asian populations.

Age estimation from human skeletal remains is an important step to reconstruct a biological profile such as age, ancestry, sex, and stature, which could assist in personal identification of unknown skeletal remains. Cranial sutures have long been studied for their age-related closure. Some researchers suggested they can be applied for age estimation, while others provided a caution according to their unreliability and wide estimated age ranges. However, until now, forensic anthropologists still attempt to investigate the best way of estimating age at death from cranial suture closure because the skull is usually found at the crime scene due to its distinct appearance and endurance to postmortem insults.

For these reasons, a study of age estimation from cranial suture closure in a Thai population was conducted to develop an age estimation model from cranial suture closure. A total of 48 cranial sutures from 296 dry crania belonging to Thai individuals were visually examined for their closure. According to the results, 17 age estimation models applying closure of the 17 sutures are proposed. The 17 age estimation models were derived from cumulative probit regression analysis. They were developed from 17 sutures: 13 endocranial sutures, 3 ectocranial sutures, and 1 maxillary suture. Thirteen endocranial sutures were six parts of coronal, four parts of sagittal, two parts of lambdoidal, and one occipitomastoid. Three ectocranial sutures were coronal pterica, pterion, and sphenofrontal. One of the maxillary sutures was posterior median palatine. Sensitivity and specificity of these 17 models were also analyzed. Most of the models revealed surprisingly high sensitivity (100%) in a group of young adults (<35 years), but relatively low specificity. This suggested that all of these models would work well if the age of unknown remains were narrowed down to young adult. In addition, the models derived from coronal bregmatica on the right side (80% for both sensitivity and specificity) and sagittal bregmatica provided relatively high sensitivity and specificity in a group aged less than 35 years (88% and 70%, respectively). These two sutures could serve as practical age indicators.

In conclusion, the present study provides an in-depth investigation of applying cranial suture closure for estimating age at death of Thai individuals. It is important to note that it would be inappropriate to rely solely on cranial suture closure as an age indicator if other more reliable skeletal part(s) are also found. In such circumstances, consequently, cranial suture closure could be applied as a supportive age indicator.

### Forensic Anthropology, Age Estimation, Cranial Suture



## A30 Does Age Matter?! An Age Study on Maxillary Sinus Morphologies in Human Identification

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**Learning Overview:** After attending this presentation, attendees will understand the potential of using age-related morphological evaluations of maxillary sinuses for identification purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insights into age-related changes of maxillary sinus morphologies and how those can be used in human identification.

**Introduction:** Maxillary sinuses are located behind the cheekbones and are the largest of the paranasal sinuses. Pneumatization of the sinuses remains throughout the whole life of an individual and starts around the third month of fetal development.<sup>1,2</sup> Paranasal pneumatization has previously been determined using measurements, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and radiographic imaging as well as liquid injections.<sup>3</sup> Especially in forensic casework, radiographic imaging proves useful, as maxillary sinuses are visible on most dental overview images.<sup>4</sup> Pneumatization varies extremely between individuals and is influenced by age.<sup>5</sup> Furthermore, age estimation is one of the key factors adding to a biological profile aiding in forensic cases. Therefore, an understanding of age-related changes in maxillary sinus morphologies is crucial to adapt this knowledge for biological profiling.

The aim of this study is to assess and classify age-related changes on maxillary sinus morphologies and to quantify their variability for human identification purposes.

**Materials and Methods:** For this study, a total of 690 digital radiographs were collected, deriving from two collections of the American Association of Orthodontists Foundation (AAOF) Craniofacial Growth Legacy Collections Project. Within the framework of those longitudinal studies, radiographs were taken of the same individuals on a semi-annual or annual basis for the purpose of determining how the skull grows between ages 2 to 70 years. Only standardized anteroposterior radiographic images were used for evaluations.

In this study, morphological growth was evaluated using Photoshop® and SHAPE™ ver. 1.3, applying elliptic Fourier analyses. Analyses of Variance (ANOVA) statistics were applied to quantify the variability of the maxillary sinus morphologies. The analyses were all performed sex-specific and compared between left and right maxillary sinus. Individuals with pathological changes in the maxillofacial area were excluded from the study.

**Results:** Not all of the collected data have been analyzed to date. However, study hypotheses as well as intermediary results indicate significant morphological variations between each individual's differently aged sinus morphologies, as well as between age groups. Furthermore, morphological changes can be clustered into meaningful age ranges and calculations of morphological variation show potential to be used in human identification. Inter-rater reliability displays high degrees of agreement both in processing sinus morphologies as well as clustering and calculation of variation. These results combined with the frequent availability of the structure on dental overview images reveal the high potential of age-related variability assessments of maxillary sinus morphologies. Further studies will discuss the opportunity of applying age-clustered morphologies as a blueprint in real-life scenarios.

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### Maxillary Sinus, Age Study, Morphological Evaluation

## A31 The Use of High-Resolution Computed Tomography (CT) to Explore Age-Related Trabecular Change in Human Ribs

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**Learning Overview:** After attending this presentation, attendees will better understand the utility of micro-CT for visualizing the microstructural organization of trabecular bone within the human rib. This presentation will increase attendee knowledge of how trabecular bone properties vary in relationship to age throughout the medullary cavity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing practitioner knowledge of age-related bone loss in the human rib that can be applied to research design and interpretation of age at death in forensic casework.

Rib histomorphometry is a reliable and often-used approach for estimating age at death based on the quantification of cortical bone, though recent efforts raise the question of incorporating trabecular bone in these assessments to improve reliability and repeatability. Despite this suggestion, trabecular bone's relationship to age in the rib, unlike cortical bone, has not been thoroughly examined. In the 2D sections traditionally used in age estimation, distinguishing trabecular from cortical bone in transitional areas can be difficult, whereas 3D analysis may more accurately capture this variation. The aim of this research is to use high-resolution CT to provide a more complete picture of trabecular bone microstructural changes within the rib and explore its utility for improving methods of estimating age at death. This information can help to determine the utility of using total bone area, rather than cortical area, measurements for improving the accuracy and repeatability of histological age-at-death estimates.

The sample consists of 40 males with ages ranging from 20–95 years from the modern Texas State Donated Skeletal Collection housed at the Forensic Anthropology Center at Texas State. Ribs were measured along the cutaneous surface and markers placed at 50% and 75% of the total length, beginning from the head. Ribs were scanned using a Northstar Inc. X5000 high-resolution CT system at both the 50% and 75% length zones with resolutions ranging from 35–49 microns. For analysis, 6mm Regions Of Interest (ROIs) were selected proximally to each marker and processed using Dragonfly V4.1 to isolate three trabecular Volumes Of Interest (VOIs): one each along the cutaneous cortex, the center of the medullary cavity, and the pleural cortex.

Using BoneJ, each VOI was analyzed for Bone Volume Fraction (BV/TV), Trabecular Thickness (TbTh), Trabecular Spacing (TbSp), Connectivity Density (ConnD), and Degree of Anisotropy (DA), within and between the 50% and 75% ROIs. Independent sample *t*-tests or Kruskal-Wallis tests were used to compare variables within and between regions dependent on normality. Within both the 50% and 75% regions, cutaneous VOIs had significantly more BV/TV than the medullary and pleural VOIs. There was significantly greater ConnD for the cutaneous VOIs than pleural VOIs in both the 50% and 75% regions, while there was significantly less TbSp in the cutaneous VOIs than either the medullary or pleural VOIs for both regions.

Between the 50% and 75% ROIs, the cutaneous and medullary VOIs at 50% had significantly more BV/TV than at the 75% region. The pleural VOIs showed no significant differences in BV/TV between locations. Overall, the 50% region has significantly more BV/TV than the 75% region ( $p = 0.002$ ). Regression was used to examine the relationship of BV/TV with age and found that BV/TV has only weak correlations with age at both the 50% ( $R^2=0.088$ ) and 75% ( $R^2=0.159$ ) regions. Additionally, at the 50% region, both TbTh and DA are significantly larger than at the 75% region. Neither ConnD nor TbSp showed significant differences between the 50% and 75% regions.

Contrary to expectations, 3D quantification of trabeculae did not elucidate a strong relationship between trabecular bone loss and age in the rib. This may be a result of excessive resorption on the endosteal border leading to trabecularization of the cortex, so that subsequent decreases in cortical bone actually lead to an increase of trabecular bone, which potentially leads to trabecular conservation throughout life. If this trabecular conservation exists, then these findings suggest that in the rib cortical, assessment alone may be enough to account for bone loss with age in histomorphometric analyses, though further work is needed.

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### Micro-CT, Age-at-Death, Trabecular Analysis

## A32 An Age-at-Death Estimation in a Contemporary Australian Population Using Femoral Histomorphometry

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**Learning Overview:** After attending this presentation, attendees will: (1) understand how bone remodeling is reflected in bone microstructure and can be used for histomorphometric age-at-death estimation; (2) appreciate the importance of population-specific standards for age-at-death estimation; and (3) appreciate the importance of sample size in relation to error and accuracy rates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for population-specific standards derived from larger reference samples. Increased understanding of the relationship between sample size and standard error will serve to improve the accuracy of histological aging methods.

Histology is an alternative approach for forensic age-at-death assessment, particularly in fragmentary remains. Bone remodeling can be assessed based on the premise that the rate of bone turnover increases as an individual ages. Histomorphometric studies use measurements of secondary osteons and their respective features to formulate age-at-death regression models. To further understand potential applications and inherent limitations of histomorphometric approaches in a forensic context, the present study examines existing histomorphometric age-at-death methods as applied to a modern Australian population.

Microradiographs from 215 femoral mid-shaft cross-sections (117♂; 98♀) were obtained from the Melbourne Femur Research Collection (MFRC) at the Melbourne Dental School; recorded chronological age at death is 18–97 years of age (pooled  $\bar{x}$ =56.1, SD=22.8; ♂  $\bar{x}$ =56.2, SD=21.8; ♀  $\bar{x}$ =56.0, SD=24.0). Twelve variables are measured across six anterior 1mm<sup>2</sup> fields of view using ImageJ. Technical Error of Measurement (TEM) and Intra-Class Correlation (ICC) are calculated to test intra-observer repeatability. The age-at-death estimation formulae of: (1) Singh and Gunberg, (2) Goliath et al.; and (3) Keough et al. are applied; stepwise linear regression is used to develop population-specific formulae.<sup>1-3</sup> The relationship between estimated and actual age at death is statistically quantified using the Standard Error of the Estimate (SEE).

The TEM values were within acceptable limits ( $r_{TEM}$ <5%,  $R$ >0.8) and ICC values were considered “excellent” (>0.9). Pooled standard error values for the existing methods were all in excess of ±20 years (range: ±22.3–22.6 years) and generally more accurate for males (SEE ♂±21.6–24.3 vs. ♀22.6–25.0 years). Population-specific modeling resulted in increased accuracy (pooled: ±19.8–22.0 years; ♂20.4–21.4 years; ♀19.4–23.0 years). When the study sample was split by age group, all methods demonstrated increased accuracy for the 35–50 year and 51–74 year age groups, with SEE values of ±10.9–21.6 and ±8.9–14.2 years for the existing age estimation methods, and ±11.2–12.6 and ±10.3–12.4 years (for the 35–50 years and 51–74 year age groups, respectively). Further examination is needed to determine the exact cause of the inaccuracies for the two remaining age groups (18–34 years and 75+ years) and any differences in bone remodeling in younger and older individuals that may be the cause. These results clearly demonstrate the importance of critical validation of existing formulae. Given the large SEE values from regression-based formulae for histomorphometric age at death, further validation of existing standards on foreign populations is necessary. Future work will include the production of population-specific standards for histomorphometric age-at-death estimation using novel variables or ratios.

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### Histomorphometry, Age-at-Death Estimation, Forensic Anthropology

## A33 Automated Techniques for Cortical Bone Histological Variable Segmentation and Image Enhancement

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**Learning Overview:** The goal of this presentation is to introduce a collection of automated image processing techniques for high-resolution imaging of cortical bone tissue. The objectives of the image processing suite include: (1) noise and artifact reduction to aid automated feature extraction; (2) streamlining researcher training by standardizing variable analysis; (3) consistent sampling techniques for reduction of inter-observer error; and (4) targeting accessible, open-source software for development of macros and standard operating procedures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing novel image processing toolkits that correct imaging artifacts and automatically extract histological variables relevant to forensic anthropology. 3D imaging techniques, including micro-Computed Tomography ( $\mu$ CT), Synchrotron Radiation micro-CT (SR $\mu$ CT), and confocal microscopy visualize 3D spaces and soft tissues within bone. Analyses focus on cortical pore and osteocyte lacunar-canalicular networks, which experience morphological change in response to aging, pathology, and drug abuse.

These image processing macros and workflows are primarily developed for ImageJ (National Institutes of Health [NIH]), Dragonfly (Object Research Systems), and DataViewer (Bruker), which are currently freely available for non-commercial use. Certain workflows also incorporate CTAnalyzer (Bruker).

The image processing automation focuses on four main objectives: (1) Artifact Correction— $\mu$ CT and SR $\mu$ CT images commonly include artifacts derived from defects in the imaging system. Workflows differentiate artifacts from real tissue spaces using pixel brightness and morphological filtering. Additionally, prior to image binarization, this study corrected for 3D tilt of the scanned bone or bone core by extracting a binary mask of the total area and aligning its skeletonized centerline; (2) Structure Segmentation—Morphological characterization of 3D tissue spaces, such as cortical pore networks and osteocyte lacunar-canalicular networks, requires conversion of the grayscale image stack into a binary image stack with pixels designated as “bone” or “space.” For  $\mu$ CT, SR $\mu$ CT, or confocal images, this study used a combination of smoothing filters, low-contrast local thresholds for pixel brightness, despeckling, and morphological closing operations to extract cross-sectional tissue spaces as 3D objects for subsequent morphological measurements. High-resolution SR $\mu$ CT datasets may pose the additional challenge of removing soft tissue visible within cortical pore canals. This study employed a cascade of morphological operations to seal pore systems that are perforated by soft tissue inclusions or by intersection with the region of interest boundaries. Confocal microscopy images, which visualize canalicular networks between osteocyte lacunae, are similarly confounded by fluorescing microcracks and soft tissue. The object analysis feature of Dragonfly uses morphometric filtering and Boolean operations for semi-automatic removal of these artifacts and further separation of osteocyte lacunae from canalicular networks; (3) Regional Analysis—Mechanical loading variation within bone cross-sections is known to alter the local distribution of cortical pore networks and trabecular bone. For  $\mu$ CT scans of femora and tibiae, this study extracted masks of anterior, posterior, medial, and lateral quadrants of both the cortical shell and the marrow cavity through automated angular rotation of the major axis. These quadrant masks facilitate regional analysis of cortical pore systems and trabecular architecture; and (4) Histological Enhancement—2D imaging with light microscopy has long been used for histological age-at-death estimation and can be further employed to interpret mechanical loading history and longitudinal changes in bone remodeling. This study modified the light microscopy setup to visualize changes in the bone tissue of rabbits following prolonged opioid exposure. The low-contrast cement lines that circumscribe rabbit secondary osteons—essential for histological age-at-death estimation—are indistinguishable on brightfield images but become visible with Differential Interference Contrast (DIC). Widefield fluorescence microscopy identifies the calcein labels administered at set timepoints during drug dosing. By combining fluorescence and DIC images, this study can isolate the secondary osteons and bands of cortical thickness that formed specifically during experimental drug dosing. This study also built a circular polarizer and analyzer, using commercially available polarizing film, to visualize Collagen Fiber Orientation (CFO). CFO is a proxy of mechanical loading in bone tissue, which may be altered by individual life history or disorganized by pathology. Regional patterns in CFO can be quantified using the Weighted Mean Gray Level (WMGL) of circularly polarized light images.

Automated image processing reduces imaging noise and inter-observer error, while improving the time efficiency of researcher training and histological analysis. Future microscopic techniques should seek automated alternatives to manual image cleaning and variable segmentation.

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### Image Processing, 3D Imaging, Histomorphometry

## A34 The Longitudinal Effects of Prolonged Opioid Use on Cortical Bone Remodeling in a Rabbit Model: Part I—Intraskelatal Variability and Regional Differences Detected Via Micro-Computed Tomography (Micro-CT)

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**Learning Overview:** The goal of this presentation is to introduce a novel longitudinal model for studying the effects of prolonged opioid exposure on cortical bone remodeling in an animal—the rabbit—that remodels its cortical bone in a manner comparable to humans. The ultimate goal is to describe how analgesic drugs, particularly morphine and fentanyl, affect microscopic structures of cortical bone used in histological age-estimation methods in forensic anthropology. Related objectives include: (1) characterizing, for the first time, 3D cortical bone microstructural changes in a rabbit opioid model; and (2) demonstrating intraskelatal and regional effects in the rabbit femur and tibia due to opioid exposure.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing how histological age-at-death estimation methods derive their accuracy from predictable cortical bone remodeling. While opioid abuse is thought to dysregulate bone remodeling, its longitudinal impact on bone microstructure has not been well defined. We must further understand the underlying biological processes to adapt histological age-estimation methods and scientific standards within the field of forensic anthropology for application to chronic opioid users.

Current evidence suggests that opioid use upsets the balance of bone remodeling toward more destruction and less formation of bone. Experimental studies have been limited by the fact that small laboratory animals traditionally used in bone research (mice and rats) do not exhibit spontaneous cortical bone remodeling, making them a poor animal model for this subject. Thus, rabbits were selected as an animal model system since they have a shortened remodeling period and relatively quick skeletal maturation. Male New Zealand White rabbits were divided into three groups of seven animals each: morphine, fentanyl, and control. Following the acclimation period, the experimental treatments for the opioid groups (morphine and fentanyl) were initiated and continued for eight weeks. All animals underwent bi-weekly subcutaneous injection with a bone-labeling fluorochrome, calcein, to facilitate *ex vivo* dynamic histomorphometry following euthanasia. It was hypothesized that opioids would significantly alter bone porosity and pore morphometry, producing substantial alterations to bone microarchitecture.

Cortical porosity was visualized in mid-shaft femora and tibiae using a SkyScan 1172 laboratory micro-Computed Tomography ( $\mu$ CT) system at The University of Akron's National Polymer Innovation Center. An imaging workflow was developed that included a 5.49 $\mu$ m pixel size, 100uA, medium camera selection, and aluminum filter. A proprietary image processing suite automatically processed all  $\mu$ CT data through macros written for ImageJ (National Institutes of Health [NIH]) and CTAnalyzer (Bruker). Workflow involved tomographic reconstruction, anatomical orientation, longitudinal alignment, extraction of total area and cortical area masks, and low-contrast filter extraction of the pore network. Pore morphometric variables, broadly associated with pore density, volume, connectivity, orientation, and cross-sectional geometry of the cortical area were assessed using CTAnalyzer.

Opioid exposure desensitizes skeletal elements to localized mechanical demands, which control intraskelatal variation in pore morphometry in healthy animals. While femoral porosity significantly exceeded tibial porosity in controls, this intraskelatal variation was silenced in morphine and fentanyl groups. This was due both to increased tibial porosity and decreased femoral porosity under drug treatment. Within the tibia, both fentanyl and morphine rabbits possessed increased total percent porosity and pore volume compared to controls. Morphine rabbit tibiae further developed individual pore systems that were significantly larger in diameter, longer, and more highly branched. The femora of morphine rabbits concurrently displayed a significant decrease in these same metrics, compared to controls.

The effects of opioids on cortical porosity were regionally concentrated in both rabbit femora and tibiae. Elevated porosity in the tibiae of opioid rabbits was localized in the anterior (fentanyl) and lateral regions (morphine and fentanyl). In the femur, decreased porosity in both morphine and fentanyl groups was significantly concentrated in the anterior and medial regions. In the tibia, drug treatment additionally silenced regional variation in metrics of pore size, pore density, and pore branching complexity. As in the whole bone analysis, drug treatment appeared to physiologically desensitize aspects of pore morphometry that were normally differentiated by regional mechanical control.

Following eight weeks of opioid administration, these results confirmed the hypothesis that opioid exposure significantly alters bone porosity and pore morphometry, producing substantial alterations to bone microarchitecture. Conventional histomorphometry is underway that will further characterize cortical bone differences among groups. Knowledge gained from these histology experiments will provide the basis for developing powerful new histological aging techniques in forensic anthropology.

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

## A35 Group-Specific Label Use in Anthropology: A Review of the *Journal of Forensic Sciences*, 2010–2019

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for the range of group-specific term use by anthropologists in forensic research in the *Journal of Forensic Sciences* from 2010 through 2019.

**Impact on the Forensic Science Community:** Attendees will gain a better understanding of the implications for the group-specific terminology used by anthropologists in its influence of the medicolegal community and anthropology as a discipline.

Race is defined as a self-ascribed, cultural construct. From this, we appreciate that it is distinct from biology (i.e., not pre-determined by our genes) and it is learned (i.e., a product of our cultural context). Forensic anthropologists instead employ the term “ancestry” to ascribe biological meaning to unidentified skeletal remains with the understanding that this term is intended to be a more accurate biological reflection of broad geographic patterning produced by natural selection over evolutionary time. However, the additional group-specific terminology used in case reports and publications has repercussions for both academia and forensic practice. The medicolegal community and law enforcement must be able to interpret research and reports in a racialized world often outside of academic understanding and consideration.

In this study, 541 articles from the *Journal of Forensic Sciences* from 2010–2019 were reviewed for use and type of group-specific labels. Evaluated research was categorized as “Anthropology” or “Physical Anthropology” or cross-listed with these categories. “Group” synonyms assessed include: “ancestry,” “race,” “population,” “descent,” “sample,” “origin,” and “ethnic/ethnicity. Group-specific labels evaluated include: “African American,” “European American,” “Africa/n,” “Europe/an,” “Asia/n,” “Caucasian,” “black,” “Black,” “white,” “White,” “Hispanic,” “Latin/x/o/a,” and “Native American” as well as notation of country-specific labels. Terms were marked as present if they were used in the description or evaluation of the data in the given study and not marked present if used in discussion of research from other authors or as part of a literature review as it is assumed those labels and/or language may be mimicking the original citation.

Of the 541 anthropology-related articles, case studies, and technical notes examined, 216 (39.9%) employed at least one group synonym and group-specific label. From 2010–2019, group synonyms and group-specific term use ranged from 29.6%–51.0% per year. Group synonym use by article results consisted of: ancestry (67.7%), race (18.5%), population (81.9%), descent (15.2%), sample (88.4%), origin (22.7%), and ethnic/ethnicity (28.7%). In most articles, more than one of these terms are used to describe the data with only ten (5.5%) employing only one term throughout. For research that employed the group labels “B/black” and/or “W/white” (78.7% of total sample), 35.9% used lower case “b” and/or “w” and 64.1% using capitalized group labels. For research referencing Hispanic and/or Latin/x/o/a groups (26.3% of total sample), 82.5% used the label “Hispanic,” 17.5% referenced Latin/x/o/a, with 14% employing both sets of terms in description or background explanation of the data.

As a discipline, forensic anthropology should consider how group-specific terminology is employed in academia as well as outside and the implications for our efficacy to the medicolegal community. Since the remains that we evaluate are often devoid of cultural context, we should strive to employ group-specific labels that are a reflection of defensible biological and evolutionary principles and use the most tentative prose when employing culturally laden terminology that cannot be justified as self-ascribed.

### Forensic Anthropology, Ancestry, Race

## A36 Population Variation in the Occurrence of Midline and Canine Diastemata

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**Learning Overview:** After attending this presentation, attendees will better understand the population and sex differences in the occurrence of central and canine diastemata. This dental variation manifests as a measurable gap between the central maxillary incisors or on either side of the canine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering insight into the degree of population and sex variation in dental diastemata. This research also highlights inter-trait correlation, which could be used in additional research to study the genetic and environmental impacts on these traits. By using modern skeletal collections for this research, the findings can contribute toward enhancing ancestry estimation methods in forensic casework and illuminating variation in these populations.

The variety of dental morphological traits allows researchers to explore population variation resulting from microevolution and varying population histories. There are a handful of methods to evaluate ancestry using dental morphological traits; however, these methods only incorporate a few dental traits, and these traits may have large observer error.<sup>1-3</sup> The goal of this study is to investigate population variation of a well-studied morphological variant—dental diastema. This trait as both a midline (i.e., between the two maxillary incisors) and canine (on either the mesial or distal side of the canine) has been extensively studied in the biological anthropological literature, which has shown the diastema trait to have documented population variation and a potential genetic component to expression.<sup>4</sup>

This study uses a large sample of data collected from documented modern skeletal samples from the United States, South Africa, and Japan ( $n=647$ ). Samples include the donated collections from Texas State University; University of Tennessee, Knoxville; University of Pretoria; Witwatersrand University; Stellenbosch University; and Chiba University. Statistical analyses explore ancestry and sex differences as well as correlation between the traits. All analyses were conducted in Statistical Package for the Social Sciences (SPSS) version 26. Diastemata were scored at the maxillary central incisors and around the right and left maxillary canines. Traits were scored according to the system outlined by Pilloud (4), which differentiates between absence (score of 0: gap < 0.5mm), low-grade (score of 1: gap between 0.5 and 1.49mm), and high-grade (score of 2: gap  $\geq$  1.5mm) diastemata.<sup>4</sup>

Results indicate no sex-based differences in diastemata presence. Population differences were found in the central incisor and left and right canine diastemata in evaluations of the raw and dichotomized data ( $p < 0.05$  in all cases). Central diastemata were most common among American Black and South African Black samples, and a high diastema grade of 2 was most common among the South African Black sample. Left and right canine diastemata were most common among the South African Black sample, and scores of 2 were uncommon. The central diastema was not largely correlated to the left canine ( $r_b = 0.413$ ) or the right canine ( $r_b = 0.377$ ). However, the left and right canine diastema were found to be positively correlated ( $r_b = 0.887$ ).

While it has been critiqued that modern orthodontia could have an impact on the occurrence of diastemata, orthodontics is a relatively recent phenomenon that is not a global practice. Based on these data, canine diastema of the maxillary central incisors and canine may be used as part of the methods to evaluate population variation and ancestry in a forensic context. Further, in another study, it was found that there was high rater agreement in the scoring of the canine diastema (kappa between 0.641 and 0.789), thereby making this trait of potential utility in forensic anthropology.

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3. Pilloud M.A., Adams D.M., Hefner J.T. Observer error and its impact on ancestry estimation using dental morphology. *Int J Legal Med.* 2019;133:949-62.
4. Pilloud M.A. Technical Note: The definition of new dental morphological variants related to malocclusion. *Dental Anthropology.* 2018;31(1):10-8.

### Dental Anthropology, Ancestry Estimation, Sex Estimation

## A37 Fluctuating Asymmetry: A Craniofacial Comparison to Better Understand Central American and Mexican Migration

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**Learning Overview:** After attending this presentation, attendees will better understand the biological consequences of socioeconomic stress on the human skeleton.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the utility of fluctuating asymmetry as a measure of environmental stress associated with socioeconomic disparities.

Decades of United States policy, intervention, and local political corruption have contributed to the deteriorating socioeconomic conditions in Latin America, which have led to mass exoduses of Central Americans and Mexicans seeking refuge in the United States. In particular, the escalation of violence in the “Northern Triangle” countries of El Salvador, Guatemala, and Honduras has given people no choice but to flee to the United States despite the risk of violence and death along the journey. Since the implementation of United States border enforcement policies beginning in the 1990s, migrants seeking refuge in the United States have been further confronted with barriers that limit their movement, resulting in thousands of migrant deaths along the United States-Mexico border.

Deteriorating socioeconomic conditions in migrant home countries have created environmental stressors that affect physical development. The inability for humans to buffer environmental stress during the critical period of physical development can result in skeletal manifestations of stress, including Fluctuating Asymmetry (FA)—deviations from perfect bilateral symmetry. Previous research has shown associations between environmental stress and Socioeconomic Status (SES)—the attainment of income, occupation, and education. With SES as the focus, the present study used craniofacial FA as a measure of environmental stress in Central American and Mexican migrants. Through craniofacial FA, the present study explored the relationship between environmental stress and push factors for United States migration.

Using a Microscribe® 3DX digitizer, 22 craniofacial landmarks were collected from 678 individuals from three skeletal samples: Operation Identification (OpID) (M = 107, F = 59), the Pima County Office of the Medical Examiner (PCOME) (M = 247, F = 36), and the University of Tennessee William M. Bass Donated Skeletal Collection (BDC) (M = 166, F = 63). The skeletal samples were classified as Central American, Mexican, and United States resident, respectively. The BDC sample was used as a base measure of SES, as these individuals were assumed to be of higher SES, experiencing less environmental stress due to their access of resources in the United States.

Craniofacial landmarks were entered into MorphoJ to generate Mahalanobis FA scores, which were then compared using a Two-way and One-way Analysis of Variance (ANOVA). Developmental timeline graphs were created for identified OpID Mexican (8), El Salvadorian (10), Guatemalan (11), and Honduran (3) individuals, overlaid with timelines of major events (i.e., North America Free Trade Agreement [NAFTA] of 1994, Mexico’s 1980s financial crisis, Illegal Immigration Reform and Immigrant Responsibility of 1996 Act, El Salvadorian Civil War 1980–1992, and Guatemalan Civil War 1960–1996), which have shown to have had major socioeconomic impacts in Central America and Mexico. The temporal relationships between developmental timelines and major events were useful in assessing the relationship between environmental stress and levels of FA. FA statistical analysis and temporal relationships were further examined to assess the relationship between environmental stress and push factors for United States migration.

FA statistical analysis showed that Mexican migrants had higher levels of FA (M = 5.03) compared to Central American migrants (M = 4.75,  $p = .014$ ) and the United States resident group (M = 4.74,  $p = .005$ ), who had similar levels of FA. The results suggest that Mexican migrants may be of lower SES, experiencing more environmental stress during physical development. Mexican developmental timelines demonstrated six temporal relationships with either the 1980s financial crisis, NAFTA, or both. Statistical analysis and temporal relationships suggest that the socioeconomic costs of both events may have contributed to the higher levels of FA in the Mexican migrant sample. Overall, Central American developmental timelines demonstrated temporal relationships with either civil war, immigration reform, or both. Considering the results from FA statistical analysis, temporal relationships, and previous research, the lower levels of FA in the Central American migrant sample may reflect how violence affects the overall population, driving individuals from all SES’s to flee from the “Northern Triangle.”

The present study demonstrates a relationship between environmental stress and historic Mexican economic migration push factors. The results further show that violence continues to act as a decisive and forceful factor for Central American United States-bound migration.

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### Fluctuating Asymmetry, Environmental Stress, Migration



## A38 Vertebral Neural Canal (VNC) Dimensions in Contemporary Subadult Samples: Indicators of Stress, Population Variation, or Both?

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**Learning Overview:** VNC measurements are historically thought to be an indicator of biological stress or Socio-Economic Status (SES). After attending this presentation, attendees will appreciate that both population affiliation and socio-economic backgrounds may be embodied in VNC dimensions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advocating caution on the interpretation of “traditional” skeletal indicators of stress in contemporary skeletal remains.

Forensic anthropologists have recently started to incorporate bioarchaeological indicators of stress into the estimation of the biological profile. Small VNC dimensions are common indicators of biological stress or low SES. VNC growth ceases during late childhood and, therefore, the impact of these negative factors is “locked in” as permanent skeletal markers thereafter. The goal of this study was to analyze the relationship between VNC size, SES, and population affiliation in contemporary subadult samples to provide a better understanding of the variation presented by this skeletal indicator.

Antero-posterior and transverse VNC diameters and the ratio of these diameters of eight vertebrae (thoracic 10 to lumbar 5) were measured on five samples of dry and virtual skeletal remains. The dry skeletal remains were from Colombia ( $n=28$ ) and included individuals from birth to 22 years. The virtual vertebrae were reconstructed from computed tomography scans of individuals from France ( $n=484$ , birth to 15 years), the Netherlands ( $n=23$ , birth to 15 years), Taiwan ( $n=31$ , birth to 16 years), and the United States ( $n=838$ , birth to 20 years). Each country is characterized by two socio-economic parameters: the Human Development Index (HDI) as a measure of life expectancy, education, and standard of living, and the Gini coefficient as a measure of inequality. Principal Component Analyses (PCA) were run to visualize the variation of VNC variables according to age, sex, socio-economic indicators, and country of origin. Discriminant Function Analyses (DFA) were run on the raw data to classify individuals into socio-economic level and population. To account for missing data, sequential nearest-neighbor imputations were done on subcategories of vertebrae and countries (Th10–L4 for Colombia, France, and the United States, and L4–L5 for all populations).

PCAs captured 83.4% of the variation in VNC for size (diameters) and 79.0% for shape (ratios) with the first two dimensions. DFA results showed that the Colombian sample clearly separated from the others for all variables and vertebrae according to HDI. Classification accuracy using VNC measurements was highest for HDI (94.00%–99.00%, two HDI groups), followed by Gini (58.00%–66.00%, three Gini groups) and population (38.00%–five groups/all populations, 66.00%–three groups/France, the United States, and Colombia). Ratios did not classify individuals as well, with inconsistent accuracies ranging from 18.00% (population, L4 and L5 of all five samples) to 71.00% (HDI, Th10 to L4 for three samples). Classifications into sex or age categories did not yield high accuracy rates in either size (45.00%–58.00%) or shape (37.00%–58.00%) of the vertebrae. Although classification accuracies were moderately high at best, these findings indicate that VNC size may be more sensitive to socio-economic factors than population affiliation. However, sampling and the differing number of groups for each set of analyses could have impacted the results.

The Colombian sample is the only sample that presents with high inequality (high Gini index) and has the lowest HDI (High) compared to the other four samples (Very High). This, as well as the sample’s small size ( $n=28$ ), may explain why accuracy rates for classifying according to HDI were the highest (94.00%–99.00%). It is also the only sample composed of individuals with a Mestizos ancestry (i.e., a particular mix of indigenous and European backgrounds). As such, and despite these higher classification rates into HDI compared to population or Gini categories, it remains unclear whether the specific ancestry composition or socio-economic level of the Colombian individuals is the causal factor behind their smaller VNC size. Results of the current study serve as a cautionary tale to warn forensic anthropologists that skeletal indicators first need to be compared to extensive, verified, and varied reference data and that statistical analyses need to account for imbalanced samples, missing data, and categories for classification before drawing any conclusions concerning individual life histories (e.g., past episodes of stress) or population affiliation.

### Human Variation, Socio-Economic Status, Vertebral Canal

## A39 Integrating the Biocultural Profile Into the Identification Process at the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory

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**Learning Overview:** After attending this presentation, attendees will understand the effective role of the biocultural profile in the process of identification for unidentified human remains at the LSU FACES laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of methods and the benefits of incorporating a biocultural profile in forensic anthropology case reports.

Forensic anthropologists assess the biological profile, including estimations of age, sex, and ancestry, to assist with the identification of unidentified human remains. However, the biological profile provides a limited representation of an individual's identity and may hinder the process of identification where social identity does not align with phenotypic expression (e.g., gender versus sex, race versus ancestry). By expanding the biological profile to include information that can be socially and culturally contextualized, the possibility of identification is increased.<sup>1,2</sup> The biocultural profile is used by forensic anthropologists at the LSU FACES laboratory to represent the integration of cultural, socioeconomic, and geographical information with biological assessments to assist with the process of identification. Case reports at the LSU FACES laboratory that utilize biocultural information render a more comprehensive profile capable of facilitating recognition for an otherwise abstract, biological description of unidentified remains. For long-term unidentified cases, the biocultural profile adds the potential for points of congruity between biocultural information in case reports and biographical information from missing persons profiles in the Louisiana Repository for Unidentified and Missing Persons Information Program (a database administered by the LSU FACES laboratory). Ultimately, the biocultural profile provides additional information associated with an individual and, thus, increases the possibility of profile recognition by linking biocultural data of unidentified remains with the sociocultural recognition of agencies and the public.

Attendees will be presented with examples from the LSU FACES laboratory of the identification process for cases that were resolved with the help of biocultural information from January 2015 to May 2020. Additionally, a case study regarding the significance of cultural accoutrements in the identification of decedents that were displaced from a cemetery during a natural disaster is discussed.<sup>3</sup> Results include an overview of patterns observed in summary statistics of biocultural data collected from LSU FACES laboratory case reports that assisted with identification (e.g., tattoos, cultural dental modifications, clothing, personal items, and recovery location). Of the 104 identified cases, 48 involved a broad range of biocultural identifiers that assisted with putative and forensic identification (approximately 46% of cases). The remaining 56 cases consisted of those where anthropologists assisted with DNA sampling and postmortem dental X-rays, Combined DNA Index System (CODIS) match notifications, and trauma-only analyses.

The purpose of this presentation is to discuss how inclusion of a biocultural profile generates information that can lead to a positive forensic identification and facilitates a better understanding of the interaction between social identity and biological expression. The success of incorporating a biocultural profile in the process of identification for forensic cases at the LSU FACES laboratory suggests that cultural and social components are important not only to an individual's identity, but also to how a person may be identified by both law enforcement agencies and the public.

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3. Listi G.A., Halling C.L., Seidemann R.M., Wilson T.V. Forensic Anthropology and Identification in Natural Disaster-Related Cemetery Disruptions. *Proceedings of the American Academy of Forensic Sciences, 71<sup>st</sup> Annual Scientific Meeting, Baltimore, MD. 2019. A98.*

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### Forensic Anthropology, Biocultural Profile, Identification



## A40 Caseloads in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will have an appreciation for the average amount and types of cases analyzed by forensic anthropologists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the factors that most affect forensic anthropology caseloads as well as the need for increased outreach to better utilize forensic anthropology in medicolegal death investigations.

Forensic anthropology exists largely to provide answers to questioned material in the medicolegal death investigation system. Thus, one could argue that the subsequent presentation of analytical findings via reporting and testimony is the ultimate goal of all forensic anthropology. However, forensic anthropologists work in diverse contexts. Some individuals are employed full time in applied positions. Others are employed full time in academia, assisting with casework on a contractual or volunteer basis. This project attempts to examine the average amount and types of casework performed by forensic anthropologists as well as the factors that have the greatest impact on the number of cases analyzed.

Data are from an online survey hosted by Western Carolina University and approved by the Institutional Review Board of this institution as well as that of the University of Nevada, Reno. The survey had a total of 364 respondents who, via self-reporting, had performed forensic anthropology casework or considered themselves qualified to do so. Not all respondents provided an answer to every question, and this project only used the subset of individuals who had responded to each question.

First, in order to examine factors that had the greatest impact on the average number of forensic anthropology reports written, a Random Forest Model (RFM) was used. This RFM found that the variables with the greatest impact on estimating the average number of forensic anthropology reports per year were (in descending order): number of fieldwork reports per year; primary current employment status; total number of cases analyzed; institution of doctoral degree; number of reports that include both field and laboratory components; and number of fieldwork cases you've participated in (in any capacity).

For current employment status, there were clear divisions between applied and academic jobs. The greatest number of forensic anthropology reports were written by individuals employed at the Defense POW/MIA Accounting Agency (DPAA) (GS anthropologists=20, median=17.5, Standard Deviation [SD]=18.1; contract anthropologists =32.4, median=30, SD=30.8), followed by forensic anthropologists employed at Coroner/Medical Examiner (C/ME) offices (23.7, median=20, SD=19.7), and finally forensic anthropologists employed in academia (7.9, median=3, SD=13.6).

For reports, on average, forensic anthropologists write two field reports per year (median=2, SD=6.4). When examining number of reports based on human remains (not fieldwork or medicolegal significance), forensic anthropologists averaged 13 reports per year (median=3, SD=20.9). When considering total human remains reports written compared to years of practice, forensic anthropologists averaged 22 reports per year (median=8, SD=63.7). Note, testifying was rare in this sample, with forensic anthropologists averaging three instances of testifying during their career (median=0, SD=8.6). Additionally, when considering report types, the most common types were: medicolegal significance (34.5%); complete skeletal analysis (30.8%); trauma analysis (13.4%); biological profile (10.2%); radiographic comparison (8.4%); and postmortem interval estimation (2.7%). Report type also varied by employment.

For institution of doctoral degree, individuals from the University of Tennessee-Knoxville had the greatest number of cases, followed in descending order by: University of Florida, The Ohio State University, and Michigan State University. While certification by the American Board of Forensic Anthropology (ABFA) was not a factor in the RFM, ABFA-Diplomates had much larger numbers of case reports overall; however, when examined by year, ABFA-Diplomates did not have significantly greater caseloads.

This study provides baseline data on the amount and types of casework analyzed by forensic anthropologists. Overall, forensic anthropologists write relatively few reports per year, particularly those employed in academic positions; additionally, testifying is very rare. However, for both casework and testifying, there is a large amount of variability between practitioners. As forensic anthropology continues to be professionalized, we should consider the relative importance of the types of reports being authored and balance our education, training, and research in these areas appropriately. The relatively low numbers of forensic anthropology reports suggest forensic anthropologists are underutilized; this indicates that forensic anthropologists must be greater advocates for our profession and our contributions to death investigations.

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### Caseload, Employment, Education

## A41 The Impact of Forensic Anthropology Manuscripts in the *American Journal of Physical Anthropology*

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**Learning Overview:** After attending this presentation, attendees will have learned the frequency and impact of forensic anthropological manuscripts in the *American Journal of Physical Anthropology* (*AJPA*).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the type of forensic anthropology manuscripts published in, and thus valued by, the *AJPA*, a flagship journal for biological anthropological research.

Dissemination of research is essential to any scientific discipline. In forensic anthropology, advances in methodology improve identification efforts, and the validation of such methodology ensures the discipline maintains *Daubert* compliance. In accordance with *Daubert*, the standard for peer-review necessitates publication in suitable journals, but until recently no such journal existed solely for forensic anthropological research. In particular, the *AJPA* serves as a prestigious publication venue due to its high publication count, averaging over 200 manuscripts per year since 2010, and high(er) impact factor (2.414). However, while the *AJPA* has been at the forefront of biological anthropological research since its inception in 1918, forensic anthropology's position is questionable. Forensic anthropology is only one small part of biological anthropology, focusing on individuals rather than populations and adhering to *Daubert* standards of validation not required by the rest of the discipline. Understanding how these differences may affect the structure of forensic anthropological manuscripts published in the *AJPA* is crucial. The primary purpose of this study was to assess the presence, impact, and structure of forensic anthropological articles in the *AJPA* to ultimately determine whether the *AJPA* should remain a pivotal journal for forensic anthropological research.

Descriptor data (e.g., manuscript titles, authors, keywords, citation counts) and abstracts for all research articles in the *AJPA* were obtained using the “rvest” package in R. Forensic anthropology articles were identified using the keyword *forensic* to assess presence over time and the average citation count. Text analysis in R using the “tidytext” package assessed word frequency, correlations, and predictors (via term frequency-inverse document frequency (tf-idf) scores). A second sample of abstract text from all research articles published in the journal *Forensic Anthropology* (*FA*) was used as a neutral control for comparison between forensic and non-forensic articles in the *AJPA*; these data underwent the same text analysis procedures.

Results suggest that while the *AJPA* is respected as a prestigious publication venue in forensic anthropology, forensic articles make up less than 2% of all *AJPA* articles. Interestingly, forensic anthropology manuscripts accrue relatively large citation counts, representing 13% of the most-cited *AJPA* articles and making up the top three most-cited articles in the *AJPA*, overall. When word frequency is assessed, non-forensic articles in the *AJPA* were characterized by: “age,” “data,” “populations,” “study,” and “human” most often, while articles from *FA* were characterized by “forensic,” “individuals,” “remains,” “age,” and “skeletal.” Forensic articles in the *AJPA* focus on “age,” but are similar to the *FA* manuscripts through commonalities such as “forensic,” “individuals,” and “skeletal.” These word frequencies reflect a broad focus on human populations in biological anthropology and a more specific focus on skeletal individuals in forensic anthropology. Next, tf-idf scores were calculated for non-forensic *AJPA*, forensic *AJPA*, and *FA* articles. Of the top ten selectors in the non-forensic *AJPA* articles, four relate to primates and three to dentition. In contrast, the *FA* article selectors follow a strong trauma focus, with “fractography,” “kerf,” “saws,” and “kerfs” among the top ten. Forensic *AJPA* article selectors are heavily methodologically focused, featuring words such as “ages,” “pronasale,” “race,” “fusion,” and “indicator.”

Results indicate forensic articles in the *AJPA* align with the overall forensic anthropological focus on methodology and validation, but are more applicable across biological anthropology. Forensic anthropology has a minimal but impactful presence within the *AJPA*, as the majority of articles accepted focus on methodology applicable across biological anthropology rather than just to the discipline of forensic anthropology.

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### *AJPA*, Text Analysis, Publications

## A42 Consistency in Validation: Categorizing Validation Studies in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will understand the current trends in validation studies in forensic anthropology over the past five years, how validation is currently ambiguously defined, and ways these studies can be recategorized to help streamline the research process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying what is meant by the term “validation,” how these studies are currently used, and proposing a cohesive definition and understanding of this term. This presentation will suggest new categories of and descriptors for validation studies to help meet the research goals of the forensic anthropology community.

To assess the current state of anthropological validation studies, 438 articles published in the *Journal of Forensic Sciences* ( $N=261$ ), *Forensic Science International* ( $N=153$ ), and *Forensic Anthropology* ( $N=24$ ) as well as 719 presentations given in the Anthropology section at AAFS meetings between 2015 and 2020 were reviewed for the use of the term ‘validation’ in the title, keywords, or abstract.

Of the 438 articles and 719 presentations reviewed, only 37% of articles ( $N=164$ ) and 5.5% ( $N=40$ ) of presentations contained keywords, titles, or abstract information relevant to the term “validation.” None of the 1,157 reviewed articles and abstracts provided a working definition of “validation.” The Forensic Science Standards Board (FSSB) defines validation as: “a process of evaluating a system, method, or component to determine that requirements for an *intended use or application have been fulfilled*” [emphasis added].<sup>1</sup> This is also the preferred definition for the Organization of Scientific Area Committees (OSAC). More than half of the abstracts and papers reviewed instead used “validation” in the context of only the first part of the definition—testing or evaluating methods to determine their applicability to a particular context or population. Results included support for methods, as well as advice against their use.<sup>2,3</sup> Some abstracts and papers did not offer a solid conclusion as to how or whether a method should be used, but rather advocated for further development.<sup>4</sup>

Finding validation studies in the literature is also problematic. Of the 40 abstracts published in the *Proceedings* of the AAFS that dealt with validation studies, only 14 mentioned the word “validation” in their title, and only 4 abstracts listed “validation” as a keyword. Similarly, for the reviewed journal articles, only 27 had validation in the title, and 9 had validation listed as a keyword; the rest of the articles contained other keywords or abstract information that fit the definition of a validation study. In response to these findings, and in order to make all existing studies more accessible, construction of a central database is proposed.

Building an accurate database of evaluation and validation studies requires consistency in terminology. Reaching consensus on the meaning and use of the term “validation” is critical if the discipline wants to be able to categorize forensic anthropology research effectively. Bethard and DiGangi note the need for validation studies in all aspects of forensic anthropology, especially those areas most likely to be called into question during court proceedings.<sup>5</sup> To meet the *Daubert* or *Kumho* requirements, the discipline needs to be able to demonstrate that forensic anthropology methods have been tested and meet general scientific merit and discipline acceptance.<sup>6,7</sup>

To clearly identify publications that are reporting validation of a method (as per the FSSB definition), the use of the word “validation” in the title and as a keyword is recommended. For any publications that present a test of a method or subject area but do not provide support for its use in a particular context, using the word “assessment” in the title and in the keywords is recommended. These suggestions for a more standard approach would also benefit the forensic anthropology community in identifying which areas need further research and work before being considered a best practice for use in medicolegal proceedings.

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### Validation, Assessment, Forensic Anthropology

## A43 A Hierarchy of Expert Performance as Applied to Forensic Anthropology

Stephanie Hartley, BA\*, Lafayette, LA 70508; Allysha P. Winburn, PhD, University of West Florida, Pensacola, FL 32514

**Learning Overview:** After attending this presentation, attendees will understand how a newly developed model organizes and frames research on forensic anthropology decision-making.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing trends and gaps in anthropological research regarding the reliability (i.e., consistency and repeatability of decisions) and biasability (i.e., effects of irrelevant information) of analyses.

Forensic scientists historically pride themselves on their objectivity, focusing primarily on objects of research while deprioritizing human observer effects on the decision-making process. In forensic anthropology, reliability is often seen as a methodological rather than an observer characteristic, and error studies usually focus on the reliability of a particular method, rather than investigating how reliable forensic anthropologists are at using a particular method. To better understand the latter, one must first understand human cognition. The human brain relies on shortcutting techniques (e.g., categorical perception) in order to process large amounts of information. These shortcuts, while enabling effective information gathering and organization, can also introduce cognitive biases—systematic distortions of the world observed.

To organize knowledge of observer effects in a particular discipline, researchers can utilize the Hierarchy of Expert Performance (HEP), an eight-level model examining intra- and inter-observer reliability and biasability.<sup>1</sup> The first four levels of the HEP focus on the observations made by experts and the last four focus on the interpreted conclusions based on those observations. As the HEP has not yet been applied to forensic anthropology, an extensive review of the anthropological literature was conducted with the goal of creating the first forensic anthropology-specific HEP. Papers from the *American Journal of Physical Anthropology*, *Forensic Anthropology*, *Forensic Science International*, and *Journal of Forensic Sciences* ( $n=3,000$ ) and Anthropology Section abstracts published in the *Proceedings* of the annual meetings of the AAFS ( $n=1,985$ ) that matched the keywords “forensic anthropology,” “bias,” “reliability,” “cognition,” “cognitive,” or “error” were reviewed for their relevancy to the HEP. The systematic literature review was further augmented by reviewing other relevant manuscripts, academic journal articles, and edited-volume chapters (e.g., articles dealing with cognition, bias, and reliability).

The resulting forensic anthropology HEP showcases areas of strength and weakness within the landscape of anthropology research. The literature review identified many forensic anthropological publications (some matching multiple levels and therefore counted multiple times) that aimed to understand the reliability of observations and conclusions ( $n=461$ ). For example, many anthropologists conducted intra- and inter-observer error studies as a part of their overall research design ( $n=290$ ) or analyzed the reliability of observer conclusions ( $n=171$ ). In contrast, the comprehensive literature review revealed few studies that dealt specifically with the biasability of forensic anthropological observations or conclusions ( $n=20$ ). Notably, while several studies demonstrated the effect of extraneous information on anthropological morphological assessments, there was no research into these effects on anthropological metric assessments.<sup>2-4</sup> Also, the majority of research regarding biasability dealt with extraneous contextual information rather than extraneous personal information (e.g., mood, stress, and motivation).<sup>4</sup>

These results indicate areas for future forensic anthropology research—into the bias-related HEP levels in general and the biasability of metric analyses in particular. Together with the ample data on the reliability of forensic anthropology methods, these results showcase the strengths and highlight the shortcomings in current forensic anthropological research on reliability and biasability. As it continues to be populated with data, the discipline-specific HEP will influence the field’s approaches to recognizing and mitigating cognitive biases in our observations and conclusions.

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### Biasability, Reliability, Forensic Anthropology

## A44 Reliability and Biasability of Sectioning-Point-Based Sex Estimation

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**Learning Overview:** After attending this presentation, attendees will understand the effects of extraneous biasing information on skeletal measurements and anthropological sex estimates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing that, while cognitive biases do not significantly impact measurements of the humeral and femoral heads or sex estimates of those individual skeletal elements, biasing context does statistically significantly impact the overall, categorical sex estimates of skeletal assemblages.

Scientific knowledge production is not purely objective, as it often includes subjective human cognitive processes. Forensic anthropologists must be able to make observations and draw conclusions with minimal compromise by cognitive biases in order to provide reliable and accurate expert witness testimony. A recently conducted comprehensive review of the anthropological literature revealed that there have been several studies ( $n=20$ ) demonstrating the effect of extraneous information on morphological assessments.<sup>1</sup> However, no research has investigated whether anthropological metric assessments are affected by biasing context. This study was designed to assess the potential of cognitive bias in metric sex estimations.

Fifty-three trained osteologists, attendees at the 2020 *American Academy of Forensic Sciences* meeting, measured a difficult-to-classify human femur, either with or without the presence of a potentially gender-biasing photograph (a staged recovery scene with men's or women's clothing) and a strongly sexually dimorphic humerus. Participants used digital sliding calipers to measure the maximum femoral head diameter and/or vertical diameter of the head of the humerus.<sup>2</sup> They then used a sectioning-point sex estimation method to provide a categorical sex estimate for the individual skeletal element(s) and, if relevant, the combined skeletal assemblage.<sup>3</sup> The participants were randomly separated into three groups: control (given the femur only;  $n=25$ ); female bias (given the difficult-to-classify femur, a female humerus, and female-biasing extraneous context;  $n=14$ ); and male bias (given the difficult-to-classify femur, a male humerus, and male-biasing extraneous context;  $n=14$ ). To minimize potential influences on the decision-making process, the participants were not informed of the full nature of this experiment; the Institutional Review Board (IRB) -approved data collection protocols also included a "distractor" ancestry estimation component. It was hypothesized that, when exposed to extraneous biasing contextual information, participants would differ in: (1) their observations (humeral and femoral head measurements); (2) their provisional sex estimation conclusions for the individual skeletal elements; and (3) their final sex estimation conclusions for the overall skeletal assemblage.

The first two hypotheses were rejected. Standard Error Index values were low for all measurements, across all groups (0.31 to 1.70mm). Krippendorff's Alpha values were high for all sex estimates, across the groups, ranging from 0.747 to 0.847 (i.e., moderate to very consistent). Student's *t*-tests indicated no statistically significant differences in the measurements taken of the femoral heads, regardless of exposure to biasing context (all  $p$ -values  $>0.05$ ). Wilcoxon rank-sum tests indicated no statistically significant differences among the control, female-biased, and male-biased groups in their provisional sex estimates of the individual elements (all  $p$ -values  $>0.05$ ).

The third hypothesis was supported. Wilcoxon rank-sum tests indicated statistically significant differences in overall sex estimates between the female-biased group and both the control and male-biased groups ( $p<0.001$ ). In spite of the fact that the difficult-to-classify femur actually originated from a male anatomical skeleton, 78.5% of the female-biased group's conclusions matched the biasing information given (i.e., "Female" or "Probable Female"). In contrast, 85.7% of the male-biased group's conclusions matched the biasing information given (i.e., "Male" or "Probable Male"). Seventy-six percent of the control participants concluded that the remains were "Male" or "Probable Male."

These results highlight the fact that exposure to extraneous context may bias forensic anthropological sex estimation conclusions—even when those conclusions are based on reliable, standardized measurements. These findings will inform the discipline's approach to the development of protocols, guidelines, and best practice standards to mitigate the effects of cognitive bias.

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### Cognitive Bias, Metric Sex Estimation, Forensic Anthropology

## A45 The Effects of Enhanced Quality Control (QC) Measures on Forensic Anthropological Practice

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**Learning Overview:** After attending this presentation, attendees will understand the benefits of enhanced QC in forensic anthropology casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence that enhanced QC does not increase casework timelines.

Recent research indicates that implicit biases can affect forensic anthropological conclusions and argues for increased QC. In particular, cognitive neuroscientists recommend implementing blind analysis, blind Peer Review (PR), and linear sequential Unmasking (LSU) of potentially biasing case context (e.g., scene/autopsy reports). Yet, the benefits of such increased QC measures—and their potential detrimental effects on casework timelines—remain relatively unknown. This study uses a sample of 78 cases analyzed at the University of Florida’s C.A. Pound Human Identification Laboratory between 2013 and 2019, 42 undertaken using “normal” QC (non-blind initial analysis and non-blind PR), and 36 undertaken using “enhanced” QC, including blind initial analysis ( $n=36$ ), Independent Peer Analysis (IPA) ( $n=36$ ), blind PR ( $n=35$ ), and LSU ( $n=33$ ).

Findings from the biological profile categories of sex and ancestry illuminate contrasts between the samples. For the 41 “normal” reports that discussed sex, three report conclusions (7%) differed from the initial case notes, with no discernable pattern to the differences. For the 39 reports that discussed ancestry, 13 report conclusions (33%) differed from the initial case notes. Eleven of these cases were nearly evenly split between changes introducing ambiguity ( $n=6$ ) and changes reflecting greater specificity ( $n=5$ ); the other two evidenced changes from racial to ancestral terminology and vice versa. As no data on the peer reviewers’ initial case impressions are recorded in “normal” QC procedures, the role of PR in initiating these changes is unknown.

For the 34 “enhanced” cases where the analyst and reviewer independently estimated sex in the initial analysis and IPA, conclusions differed in 12 cases (35%). However, the final report differed from the initial analysis in only 5 of the 32 cases where sex appeared in the final report (16%). In most of these cases (three of the five; 60%), this change was in the direction of greater specificity, informed by the conclusions of the IPA. In one case, the initial analyst’s conclusions became more definitive in spite of a reviewer’s “indeterminate” sex estimation, suggesting influence by an undocumented factor (e.g., informal reviewer-analyst conversations). In 9 of 33 cases where ancestry was reported by both analysts (27%), the analysts disagreed on their estimates. Final reporting differed from initial notes in 6 of the 32 cases where ancestry appeared in the final report (19%). In four of these six cases (67%), the changes served to add ambiguity to an analyst’s initial conclusions. In one of the two cases where final ancestry conclusions changed in the direction of greater specificity, knowledge of the recovery context revealed during LSU was incorporated into the analysis. In the second, there was consensus between the analyst’s and reviewer’s initial reporting, but the final conclusion differed, suggesting influence by an undocumented factor.

Comparing the two samples, “enhanced” QC more frequently resulted in revisions to the final reporting of sex (16% vs. 7% in the “normal” sample) but less frequently to the reporting of ancestry (19% vs. 33% in the “normal” sample). In the “enhanced” sample, PR led to more specific sex conclusions but exerted a generalizing influence on ancestry conclusions. There was no clear pattern in the nature of changes made by analysts in the “normal” sample. In essence, “enhanced” PR enabled more specific estimates of a relatively straightforward variable (sex) and more general—potentially more accurate—estimates of a more ambiguous variable (ancestry). The clarity gained from the reviewers’ completion of a blind IPA seems to have structured the “enhanced” analyses and enabled productive dialogue between the analysts during this process. Both blind PR (with IPA) and LSU provide context for analyst decisions and render the changes made throughout analysis more transparent. Finally, the fact that the mean case turnaround time (from initiation to final report) was similar for both samples (20 weeks for the “normal” and 18 weeks for the “enhanced” samples) rejects the traditional assumption that prioritizing QC translates to longer and more cumbersome casework. Simply, the benefits of enhanced QC outweigh the negatives.

*The data for this research comprises casework performed prior to the term of Dr. Phoebe Stubblefield as CAPHIL Director. Data do not reflect the current administrative practices of CAPHIL. The authors regret that these results were not reviewed or approved by Dr. Stubblefield prior to publication.*

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### Quality Control, Cognitive Bias, Forensic Anthropology



## A46 Improving Radiographic Visualization of the Frontal Sinus for Scientific Identification

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**Learning Overview:** After attending this presentation, attendees will understand the conditions that hinder imaging of the frontal sinus and be provided with a method for enhancing the morphology of the frontal sinus radiographically.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a method of improving radiographic visualization of the frontal sinus using the radiopaque contrast medium Barium Sulfate (BaSO<sub>4</sub>) for comparative medical radiography.

Contemporary research and case studies looking at the variability and reliability of the morphology of the frontal sinus for positive identifications have demonstrated the frontal sinus to be a valuable and unique point of correspondence for positive identifications in a medicolegal setting.<sup>1,2</sup> However, due to the taphonomically altered nature of cases requiring positive identifications, capturing quality postmortem imaging of the cranium can be challenging. Autolysis of neural tissue is one of the earliest signs of decomposition, and in cases of advanced decomposition, liquefaction of the neural tissue increases radiographic attenuation, which can produce blurred features on postmortem imaging.<sup>3</sup> Conversely, in skeletonized remains or burned cases with empty or thermally damaged cranial vaults, radiographic attenuation decreases, which can cause the X-ray beam to “burn” through features of interest. With digital radiographic equipment, these imaging concerns can be remedied by adjusting the preset peak kilovoltage (kVp) and milliampere-seconds (mAs) levels. However, this “trial-and-error” method of achieving the optimal settings can be a time-consuming process, especially if the individual conducting the radiography has limited experience or the equipment does not allow custom kVp and mAs settings.

From June of 2014 through September of 2020, forensic anthropologists at Western Michigan University Homer Stryker MD School of Medicine (WMed) conducted 311 comparative medical radiographic identifications for the Office of the Medical Examiner, 116 (37%) of which cited the morphology of the frontal sinus as a point of correspondence. Considering the frequency at which the frontal sinus is utilized, this pilot study aimed to increase the radiopacity of the sinus arcades by injecting the sinus with the radiopaque contrast medium BaSO<sub>4</sub> to improve visualization of the sinus and expedite postmortem imaging.

This study was conducted at WMed in Kalamazoo, MI, on 25 anatomical donors provided by the WMed Body Donation Program. Each donor’s preliminary full body radiographic scan (conducted by donation program staff at donor intake) was reviewed to determine if the donor exhibited a frontal sinus, the approximate number and shape (smooth or scalloped) of the arcades present on each side of the midline, and to determine optimal drill point locations to access the frontal sinus. If the cranium was still fleshed, the area of skin between the eyebrows was removed utilizing a scalpel and forceps, and drill points were made using a 3.2mm round fast-cutting bur. A 20% weight-volume BaSO<sub>4</sub>-water solution was then injected into the drill points using a syringe. To reduce the likelihood of the solution following the natural sinus drainage pathway, one side of the hydraulic dissection cart was lowered so the donor’s head was lower than their shoulders. The drill points were overfilled to ensure enough of the solution was injected and spillage was wiped from the area. The donor was then re-positioned and radiography was completed using the preset cranium levels of 80kVp and 12.5mAs at a minimum 40" source-to-image distance.

This method proved to be a quick and effective way to improve visualization of the sinus, reduce the number of postmortem images taken, and standardize kVp and mAs values.

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### Identification, Radiology, Frontal Sinus

## A47 A Pilot Study on Measurement Error on 2D and 3D Images in Forensic Anthropological Applications

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**Learning Overview:** After attending this presentation, attendees will better understand the applicability of radiographs and 3D scans for metric analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comparative analysis of various mediums of imagery to be analyzed and by demonstrating the possibilities and/or limitations of taking metric measurements on different types of imaging.

The keystone of forensic anthropology is the biological profile, which is estimated from various features of the skeleton and aids in the identification of unknown individuals, consisting of age, sex, ancestry, and stature. The utilized quantitative methods rely heavily on metric data, with *FORDISC*<sup>®</sup> 3.1 being the most commonly used application. In light of the COVID-19 pandemic, as well as the general increase in electronic communications between law enforcement and forensic anthropologists, understanding the distortion and error rates associated with metric analyses on 2D models, as opposed to 3D models, becomes increasingly important. 2D and 3D imagery can complicate metric analyses due to image distortion. 2D images ultimately flatten objects in the image, making measurements in a 3D plane, such as diameter, significantly less accurate. Additionally, a poorly taken photo or radiograph can blur the necessary features on skeletal elements, and a poor perspective or composition can obscure measurement landmarks or distort perceived lengths. In the case of 3D scans, human error in scanning or post-processing can create a margin of error that must be accounted for in order to have accurate results.

The goal of this research is to compare a set of measurements taken with these three different media to those taken directly on dry bone in order to assess the gross error rate associated with digital measurement protocols and to quantify the relative contribution of each source of error to these particular media. This can assess the practicality and accuracy of utilizing metric methods via different platforms, quantify the level of accuracy and margin on error when taking measurements in a 2D plane, as well as to assess the best practices for metric methods when researchers do not have access to dry bone samples. This study hypothesized that 3D scanning will provide the most accurate measurements out of the three imaging techniques with statistically lower error rates, and radiographs will be the least accurate due to distortion with statistically higher error rates.

This study compared the error rates associated with repeated measurements of five standard variables from laboratory photographs, standard radiographs, and high-resolution 3D scans as compared to direct measurements on dry bone.<sup>1</sup> A sample of ten complete left humeri were chosen due to their consistency in regard to morphology and lack of overall curvature. Photographs and radiographs were taken anterior-posteriorly and medial-laterally to best recreate all views needed to measure with the highest level of accuracy and precision. The radiographs were taken utilizing the Konica Minolta Regius console CS-2 Version 1.30R00 Digital Imaging System and were analyzed with ImageJ software. 3D scans were taken with the Artec Space Spyder and were analyzed with the associated Artec software. For statistical purposes, the maximum and minimum diameter midshaft measurements, as well as maximum length, were taken on both views of the photographs and the radiographs to account for the flattening of the 2D image and to properly assess error rates. In total, 34 measurements were taken on ten humeri, totaling 340 metric points taken per person for three people.

The preliminary results of this study are consistent with the main hypothesis articulated above: 3D scans represent the best alternative to direct measurements on dry bone, as opposed to photographs or standard radiographs. However, the results of the study also reveal that, when properly oriented and taken correctly, radiographs and photographs can produce approximately accurate measurements, as compared to dry bone, that would not result in significant differences when utilized for morphometric analyses. Inter-observer error was lowest on dry bone and highest in radiographs.

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### Stature Estimation, Radiographs, 3D Scans

## A48 The Utility of Digital Imaging Technologies for the Virtual Curation and Metric Analysis of Skeletal Remains

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**Learning Overview:** After attending this presentation, attendees will understand that cranial measurements taken from various 3D modeling platforms are comparable to each other and to measurements taken with traditional hand-held instruments. Attendees will also gain a better understanding of the techniques used to create 3D models of skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the accuracy 3D curation and showing that these technologies are viable methods of data collection for forensic skeletal cases and collaborative research.

Virtual curation methods such as 3D laser scanning and 3D photogrammetry are exciting developments within the field of anthropology. However, outside of Sholt et. al.'s research in 2011 as well as Bridget Algee-Hewitt's and Amber Wheat's research performed in 2016, there is little work discussing the accuracy of measurements taken from these models.<sup>1,2</sup> This research adds to that body of literature. It compares the accuracy of 3D laser scanning, 3D photogrammetry, and a 3D digitizer to measurements taken in the traditional way by testing two hypotheses: (1) that measurements obtained from 3D models are comparable to traditionally derived metrics, and (2) that measurements taken on different 3D modeling platforms are comparable to each other.

To evaluate the accuracy of the 3D laser scanner, the 3D digitizer, and 3D photogrammetry, all 28 standard cranial measurements, excluding those of the mandible, were recorded nine times for three skulls ( $n=9$  for each measurement) and compared to traditional measurements. The averages and ranges for the datasets from each skull were then used to determine if there was a statistically significant difference between the different modeling technologies, and between the modeling technologies and hand-held metrics. 3D models of each skull were made by rotating them in 20-degree increments, and if areas of the 3D model were incomplete, individual images or scans of the skull were made to capture the missing area. After the models were uploaded, points were placed on each cranial landmark, and inter-landmark distances were calculated electronically. Once the outliers were removed, the data were analyzed using Levine's Equality of Variance and a two-way Analysis of Variance (ANOVA) test.

In a comparison between the measurements taken on the 3D models and the traditionally derived measurements, there was no significant difference between the digitizer ( $p = .818$  to  $1.0$ ) or the 3D laser scanner ( $p = .900$ – $.999$ ). This trend continued in a comparison between 3D photogrammetry and traditionally derived measurements ( $p = .827$ – $.996$ ). These results indicate that digital 3D models can be used as an accurate substitute to taking measurements in the traditional way, since the inter-landmark distances derived from each method were comparable to each other.

Comparisons between the different 3D modeling techniques also yielded no statistically significant results. The  $p$ -values ranged from  $.716$ – $.992$  in a comparison of the digitizer to the laser scanner, and  $.813$ – $.998$  when comparing 3D photogrammetry to the digitizer. Similar results occurred in a comparison between the 3D laser scanner and 3D photogrammetry ( $p = .868$ – $.969$ ), showing again that there was no statistically significant difference.

The results from this study demonstrate that 3D models created with various platforms are an acceptable form of digital curation, since the measurements taken on the 3D models were comparable to the measurements taken using traditional techniques. Furthermore, the different 3D modeling techniques were comparable to each other, meaning that these technologies can be used to virtually curate and share images without a significant impact on metric data collection. This research supports the use of these technologies for virtual data capture and information sharing between researchers, making future collaborations between distant universities easier, providing broader access to collections. It will also allow the opportunity for remote work when in-person work is impossible or hazardous.

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### 3D Modeling, Metric Analysis, Virtual Curation

## A49 3D Web-Based Technologies to Support Interactive Forensic Anthropology Databases

*Terrie Simmons-Ehrhardt, MA\*, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** After attending this presentation, attendees will understand the available open-source web-based technologies for enabling interaction with 3D bone models to enhance existing and new forensic anthropology reference databases and applications both online and offline.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the implementation of 3D web technologies into forensic databases to encourage interactive references, accessibility to common datasets, and enhancement of quantitative applications.

3D skeletal models provide an opportunity for anthropologists to interact with examples of skeletal variation, pathology, and trauma, to demonstrate data collection procedures, and to develop quantitative methods in place of or in support of visual methods. The availability of medical imaging databases and digitized dry skeletal specimens can improve access to skeletal variation and contribute to common reference datasets. Additionally, the sharing of generated 3D models or de-identified volumetric medical imaging data can provide method training for specific applications, while reducing duplicate digitization/rendering efforts. Despite the availability of free software such as 3D Slicer for generating 3D models from medical imaging data and other free software for interacting with 3D models, the learning curve for 3D software can be steep, and the time required to develop proficiency and to generate large numbers of models may be prohibitive to some anthropologists. Therefore, the ideal sharing mechanism for 3D skeletal models would provide a widely accessible, straightforward interface with simple tools such as multi-model visibility, cross-sectional clipping, transparency, measuring, annotations, and have cross-platform capability and portability with offline viewing options to protect data that cannot be distributed online.

Web-based technologies have progressed to facilitate customizable, open-source 3D interactivity within a browser window utilizing HTML and JavaScript™ to support common 3D model formats. Existing online 3D repositories allow for private/protected viewing, but do not include features such as dynamic clipping, measuring, or multi-model visibility. 3D Heritage Online Presenter (3DHOP) (3dhop.net) was developed to display cultural heritage resources online but also supports offline viewing and has been demonstrated by this study on both tablet and laptop with references for facial approximation, micro-Computed Tomography (CT) skeletal specimens, and full body CTs containing multiple models. Models can be in a PLY format with solid color, color mapping, or in a compressed format optimized for “streaming” large models over the web. Three.js (threejs.org) supports models in various formats as well as de-identified CT volumes or sub-volumes generated with 3D Slicer or Fiji. Although the code for these applications is open-source, 3D HTML pages do not need to live online as they also function offline utilizing a local server or by allowing the browser to read local files. Additionally, customized packages or the models or volumes can be placed online under registration-only access or provided for offline viewing via request or data repository. 3D HTML pages are also supported by R Shiny (shiny.rstudio.com) allowing quantitative applications to include 3D references/examples or for attachment of 3D models to existing applications. The cross-platform capabilities and offline viewing would also support the demonstration of 3D evidentiary models with custom viewers in the courtroom.

This presentation will demonstrate how 3DHOP and three.js can be customized for forensic anthropology applications, 3D HTML integration with R Shiny, and options for online or offline implementation.

### 3D Modeling, HTML, Virtual Anthropology

## A50 Back Together: An Innovative Procedure For Cranial Reconstruction as an Aid for Human Identification

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**Learning Overview:** After attending this presentation, attendees will better understand the potential of a new reconstructive approach to fragmented skeletal material.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new procedure to reassemble and reconstruct fragmented skeletal remains for a more comprehensive analysis

Human remains are frequently recovered fragmented from forensic contexts. Taphonomic factors and perimortem trauma and injuries can damage human remains recovered from clandestine graves. Therefore, an incomplete or broken skull can represent a challenge to the identification of an individual, osteometric analysis, and the interpretation of trauma to establish cause and manner of death.

A reconstructive approach is proposed to aid forensic experts (i.e., medical examiners, pathologists, forensic anthropologists) in achieving all the information from the human remains. This study proposes an innovative method that involves the use of reversible glue to connect the fragments. Non-permanent wax is then used to reconstruct the missing parts and stabilize the skull.

The reconstruction procedure is divided into three phases: cleaning, reassembling, and remodeling. All the phases are documented to maintain the value of the forensic evidence. The phases are carried out after a preliminary evaluation of the remains and evidence collection (e.g., trace and genetic evidence).

The bones, particularly the fractured areas, are cleaned with cold water. This preliminary step enables the expert to avoid any error caused by sediment and exogenous material in the fractures. All the fragments are classified and photographed before starting the reconstruction to guarantee a more straightforward process.

The reassembling is carried out with non-permanent reversible glue (Paraloid™ B-72 crystals mixed with acetone in a 60% solution). Consequently, if the reconstructed remains do not have enough solidity to undergo a forensic examination, some of the missing anatomical parts can be replaced with reversible wax, modeled on the missing bone's shape. The wax is a pigmented combination of beeswax, hydrated calcium sulfate, pine resin, and paraffin.

The procedures described in the present research have been extensively applied during a PhD project carried out at Liverpool John Moores University to allow the craniometric analysis of 400 skulls from two British medieval sites (Poulton, Cheshire, and Gloucester, Gloucestershire). The cranial reconstruction improved the collection from Gloucester by 69%, reassembling 76 skulls completely fragmented; while the Poulton collection has been improved by 56% (an additional 175 reconstructed skulls have been included). Furthermore, they were largely applied in different scenarios at the University of Florence, allowing the full reconstruction of skulls from archaeological excavations of different periods (Prehistoric, Etruscan, Medieval, and Historical). The success of these reconstructions has been demonstrated by the publication of the data obtained from the different sites.<sup>1-5</sup> In addition, the reconstruction approach has been applied in forensic cases that have been presented in the Italian court.

The method proposed allows a more comprehensive examination of the whole skull structure for the biological profiling of unknown individuals, as well as a better analysis of trauma and injuries, if present. Moreover, Computed Tomography (CT) and radiographic analysis can be performed on a reconstructed skull rather than on fragmented specimens; the data obtained can also be a more appropriate background for unidentified persons' facial approximations.

This research aims to propose a new reconstructive approach to fragmented skeletal material, particularly skulls, that can be damaged by taphonomic or traumatic events. This study also presents the benefits of using a pigmented wax to reconstruct missing anatomical parts to adequately analyze incomplete remains.

### Reference(s):

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2. Borrini M., Solaini P., Golloher M. Preliminary Analysis of the Pathological and Traumatic Conditions on Skeletal Samples from a Plague Cemetery of Venice. *Journal of Biological Research*, 2012. LXXXIV :220-221.
3. Borrini M., Riccadonna L., Borrini C. A possible juvenile hypochondroplasia case from the mass grave of Lazzaretto Nuovo Island (Venice). *Annali dell'Università di Ferrara*, 2014, 10 :97-101.
4. Borrini M., Donati M., Murgia C. Donati M., Murgia C. Skeletal remains from the cemetery of Lazzaretto Nuovo (Venice): A preliminary analysis. *Annali dell'Università di Ferrara*, 2014, 10 :85-90.
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### Forensic Anthropology, Skeletal Reconstruction, Taphonomy

## A51 The Application of Standardized Skeletal Inventory Methods to Fragmented Subadult Cranial Remains

*Andrea Palmiotto, PhD\*, Indiana University of Pennsylvania, Indiana, PA 15701*

**Learning Overview:** After attending this presentation, attendees will have gained awareness of the importance of critically assessing standardized adult-based skeletal inventory methods when applied to subadult cranial remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) considering the utility of methods developed for mature skeletal remains when applied to subadults, and (2) recommending modifications to existing skeletal inventory methods when applied to subadult cranial remains.

Standardized inventories are integral for accurate skeletal assessments, including estimation of the Minimum Number of Individuals (MNI) for an assemblage. While previous research suggests that standardized methods perform nominally for mature skeletal remains, they have not yet been tested on subadult remains. Because subadult remains are less developed, it is expected that morphological traits may not be easily discerned; therefore, subadult remains will be more difficult to quantify.

Because subadult skeletal development progresses in predictable stages, this research assesses standardized inventory methods for different age classes of fragmented, subadult cranial remains. Skeletal remains were examined from previously analyzed bioarchaeological collections housed at the Carnegie Museum of Natural History. The remains are associated with Early–Middle Woodland and Monongahela sites in Western Pennsylvania. They were recovered from various burial contexts in fragmented and sometimes commingled conditions. The remains were segregated and MNI was established by previous analysts using traditional techniques, including burial context, dental formation and eruption, and epiphyseal union.

For the current study, the cranial remains from 33 subadult burial features were assessed using the landmark and zonation methods. The individuals were categorized as infant (0–1 years,  $n=17$ ), young child (1–6 years,  $n=9$ ), older child (7–12 years,  $n=3$ ), and adolescent (13–16 years,  $n=4$ ). Each set of cranial remains was assessed for the presence of zones (cranial zones 1–15; mandibular zones 1–14) and landmarks (cranial landmarks #1–#52; mandibular landmarks #53–#65). The zones represent individual cranial bones (e.g., cranial zone 3, right parietal) and portions of the mandible (e.g., mandibular zone 1, left horizontal ramus), while landmarks refer to specific features on a bone (e.g., #14, right parietal eminence or #56, left mental foramen).

Among infants, the zonation method ( $n=16$ ) outperformed the landmark method ( $n=15$ ), but neither method identified all 17 infants. Duplicate left petrous portions (#45) were identified in one set of remains, suggesting inaccurate segregation or a higher MNI than previously determined. Some elements, such as parietal bones, could not be identified by zones or landmarks due to the absence of developed morphology. Additionally, small elements, such as nasal bones, palatines, and vomers, were not identified. Among all other age classes, both the zonation and landmark methods resulted in the expected MNI. Among young children, parietal bones displayed diagnostic morphology consistent with zones and some landmarks, but small elements were still not easily identified. Among older children and adolescents, developed features were identifiable, and the expected individuals were represented by multiple zones and landmarks.

While both methods can be used to inventory fragmented subadult cranial remains accurately and assist in the resolution of commingling, the zonation method permits more flexibility using criteria that may not be represented by the landmark method. However, the zonation method is more subjective and does not provide a clear way to account for overlapping portions of fragments within the same zone. The landmark method relies on bone features that may be unidentifiable or underdeveloped in subadult skeletal remains. For example, while an analyst may recognize a parietal fragment by the presence of meningeal grooves and striae, these features are not part of the landmark system. If the parietal eminence, inferior temporal line, and squamosal suture are underdeveloped, parietal fragments risk being unidentified. Additionally, several landmarks are foramen (e.g., #39, foramen magnum), which may be difficult to identify among subadults due to the absence of fusion (e.g., between the occipital condyles, #40/41 and basicranium). Therefore, several modifications are recommended for the landmark method when applied to subadult remains.

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### Subadult Skeletal Remains, Zonation, Landmarks

## A52 A Metric Approach to Human and Non-Human Species Identification of Skeletal Remains Using Machine Learning

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**Learning Overview:** After attending this presentation, attendees will understand the advantages and limitations of using basic postcranial measurements to evaluate human versus non-human remains and species identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how a few simple skeletal measurements that can be taken without extensive osteological knowledge can be used to objectively evaluate whether skeletal elements are human or non-human and estimate the non-human species.

Forensic anthropologists are routinely consulted by medicolegal and law enforcement agencies to evaluate whether skeletal remains are human, and thus of forensic significance, or non-human (i.e., animal). Most of these unknown elements turn out to be non-human, at which point anthropologists are commonly asked to identify the species. Although the question may be asked out of curiosity, a correct faunal species identification boosts agency confidence in the non-human determination and may be relevant to forensic investigations. Current macroscopic species identification methods rely primarily on visual assessments, which can be subjective and dependent on analyst experience. This study thus aimed to evaluate the utility of bone length and breadth measurements in human and non-human species identification.

Humeral and femoral maximum bone lengths, epiphyseal anteroposterior and mediolateral maximum breadths, and midshaft maximum and minimum diameters were collected or compiled from published data from humans and 26 common North American faunal species. Sample sizes varied by species, element, and measurement, but in total 16,049 measurements were collected from 436 human and 984 non-human specimens. Random forest models were run on the pooled sample for each skeletal element (e.g., pooled humerus data) using a training subsample (70% of the sample), then a testing sample (unused 30% of the sample), and correct classification rates and variable importance were recorded. For each model, 500 trees were employed, with three variables tested at each node. Humerus and femur datasets were used separately to create models to differentiate human versus non-human (all non-human samples pooled) and identify individual species. An additional model was created to test if both species and bone could be estimated from analogous measurements. For human versus non-human classification, accuracy rates were over 99% (humerus = 99.77%; femur = 99.40%). Species identification rates were similar for the femur (85.3% overall) and humerus (84.46% overall). As might be expected, misclassifications were highest among related taxa (e.g., brown bear versus black bear). Last, the correct classification for both species and bone was 82.18%, which is quite high given there are 54 response categories, making random chance of success 1.85%.

Despite the 27 species included in these analyses and some of the large variations in size and morphology within some of the species (e.g., domestic dog), the random forest models returned relatively high correct classification rates. Therefore, the use of simple postcranial metrics can assist those without extensive comparative osteology experience in identifying, or at the very least, narrowing down the possibility of species to facilitate a visual identification from other resources. The metric classifications can also be used to statistically support forensic anthropological assessments of human versus non-human, or faunal species identifications. Such metrics also have the potential to assist in the species identification of fragmented remains. Given the success rates with the humerus and femur, additional analyses are being conducted on the remaining long bone elements, with the aim of creating a freely available online tool to facilitate species identification from skeletal remains across and beyond the forensic community.

### Random Forest Modeling, Osteometrics, Species

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## A53 A Comparison of the Accuracy of Identification Between Non-Human and Human Skeletal Elements

*Kaitlyn Klein, BS\*, Erie, PA 16504; Holly A. Long, BS\*, Mercyhurst University, Stilwell, KS 66085; Summer B. Shipley, BS\*, Mercyhurst University, Erie, PA 16546*

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**Learning Overview:** After attending this presentation, attendees will understand the significance of zooarchaeology training in the identification of non-human versus human skeletal elements in potential forensic cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the importance of having a readily available forensic anthropologist with a proper background in zooarchaeology for all active crime scene units and law enforcement agencies.

Human versus non-human assessments are a common component of the caseload of forensic anthropologists, as forensic significance is often primarily based on that distinction. Without zooarchaeological training, animal remains are often initially misidentified at the scene, by law enforcement or the general public, as potentially human, frequently leading to unnecessary wastes in investigative time and resources. On the other hand, proper identification and description of reported animal remains beyond the basic human-vs.-non-human assessment serve as positive reinforcement for the reporting of suspected skeletal element findings by the public, by demonstrating to the discoverers that their reports were meaningful and received comprehensive examination and consideration. Due to these considerations, there is a consistent need of properly trained forensic anthropologists to assist in establishing the forensic significance of skeletal elements in potential cases.

Ten human skeletal elements and ten non-human skeletal elements were photographed with and without scales from six views and randomized into a singular document. The participants were asked to record their conclusions. The use of photographs in this study instead of in-person analysis is both due to the current coronavirus pandemic and because photographic identification is indeed very commonly utilized by forensic anthropologists in the initial stages of this type of consultation. The survey document was sent through email to each of the 50 participants. Seventeen of the participants were law enforcement officers with no formal human osteological or zooarchaeological training. Seventeen of the participants were Department of Applied Forensic Sciences (DAFS) first year graduate students and undergraduate students, as well as various individuals with formal training in human osteology only. Sixteen of the participants were Mercyhurst University DAFS second-year graduate students, staff, and outside practicing forensic anthropologists with both human osteological and zooarchaeological training. Each member of the study was prohibited from using outside reference materials in the identification process. After opening the document, participants were asked to view each photograph and determine if it was a non-human or human skeletal element. If able to do so, they were further asked to identify the specific bone as well as the species and side. The document was then returned, and the results were compiled for preliminary statistical analysis.

Participants without osteological training demonstrated lower accuracy rates in the identification of human versus non-human skeletal remains. Those who had formal human osteological training but no zooarchaeological had a higher accuracy in determining non-human skeletal remains from human remains. The participants who had received formal training in both human osteology and zooarchaeology had high accuracy in identifying human skeletal remains from non-human in a very timely manner. The ability of those trained in both human osteology and zooarchaeology to identify bones in more specific detail offers valuable skill and is key for crime scene analysis and reconstruction.

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### Zooarchaeology, Human Remains, Identification



## A54 Portable Laser-Induced Breakdown Spectroscopy (LIBS) for the Categorization of Human Skeletal Remains

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**Learning Overview:** After attending this presentation, attendees will understand how LIBS can be utilized as a non-destructive technique to categorize human skeletal remains in bioarchaeological and forensic anthropological contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropologists and bioarchaeologists may be able to utilize LIBS to categorize human skeletal remains through rapid elemental analysis in the field using portable instrumentation.

Bioarchaeology and forensic anthropology require the classification of unidentified fragmentary materials. In cases of mass disasters and commingling, it is important to determine whether fragments are osseous or not to aid in the process of identifying individuals. Currently, most elemental analyses of bones are conducted using portable X-Ray Fluorescence (pXRF). However, there are limitations to the elements pXRF can detect without the need for additional equipment or analysis time, specifically elements with lower atomic mass. Portable Laser-Induced Breakdown Spectroscopy (pLIBS) offers a sophisticated alternative to pXRF by using rapid laser ablation to analyze elements. With this analysis occurring at the micro-scale, the technique is non-destructive to the sample as it typically cannot be seen by the naked eye. Considering this, pLIBS could offer a superior approach for materials discrimination and human identification. This study addressed the following questions: Can individuals be distinguished from one another using pLIBS to obtain elemental signatures from bones? If so, can pLIBS be considered an improvement over current methods for skeletal analysis in bioarchaeology and forensic anthropology?

Human skeletal materials were utilized from the donated collection at the Institute of Forensic And Applied Science (IFAAS) at the University of South Florida. The sample consists of seven donors, including one set of cremains, made up of four females and three males ranging from 28 to 79 years of age. Two archaeological samples from a medieval Transylvanian skeletal collection were analyzed for comparison between modern and historical remains. Data were collected at 206 locations on 29 bones across the skeleton in order to capture the best representation of the overall elemental composition of an individual and to test for intra-individual variation. Elemental analysis was performed using a SciAps Z-300 LIBS analyzer connected to the proprietary SciAps ProfileBuilder spectral analysis software. IBM® SPSS Statistics 25 was used to perform Multivariate Analysis Of Variance (MANOVA), post hoc analyses, and Discriminant Function Analysis (DFA) on the data set. Through a combination of LIBS and multivariate data analyses, it was determined that elemental data taken from human skeletal remains could be used to differentiate each individual from another. Beyond this, donors were successfully categorized by disposition (i.e., caged, tarped, surface) based on their placement at the IFAAS facility, indicating the influence of the burial environment on sorting skeletal remains. This research provides new insights into the use of LIBS for human skeletal analysis and its ability to rapidly and non-destructively differentiate between individuals using elemental variation within bones.

### LIBS, Commingling, Forensic Anthropology

## A55 Choose Your Own (Accreditation) Adventure in the Defense POW/MIA Accounting Agency's (DPAA's) Isotope Testing Program: Part I—Sample Preparation

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**Learning Overview:** After attending this presentation, attendees will have learned about the work completed to accredit isotope sample preparation under International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025:2017 for Geographic Profiling (i.e., isotope testing) of human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by illustrating the process used by the Defense POW/MIA Accounting Agency (DPAA) Laboratory to prepare for accreditation for isotope testing—specifically sample preparation—of unidentified individuals.

The DPAA Laboratory began investigating the utility of isotope testing for identification in 2012. The technique provides information about an individual's life history, as human tissues record the isotopic signatures of ingested water and food. These signatures can and do vary geographically. At the DPAA, the program is used to refine short lists for identification, to separate commingled remains, to eliminate non-United States remains from the DNA testing stream, and to add another line of evidence for an individual identification. Initially, isotope testing by the DPAA was completed through contracts with external providers for both sample preparation and analysis.

In 2017, the DPAA Laboratory in Hawaii began developing in-house capabilities for the preparation and analysis of osseous and dental remains for the “bio” elements—carbon, nitrogen, oxygen, and sulfur. One goal was to accredit isotope testing under ISO/IEC 17025:2017, appearing on the DPAA Laboratory's Scope of Work as “Geographic Profiling.” This presentation describes the work completed in 2019 to accredit isotope sample preparation with the American National Standards Institute National Accreditation Board (ANAB). Another presentation describes work completed in 2020 to accredit isotope sample analysis.

The initial step for accrediting isotope sample preparation was site validation, which focused on facilities, equipment, and training. Facilities were surveyed and deemed adequate, with sufficient security protocols, space, lighting, and climate control. Work areas were determined to be free of environmental influences that could adversely influence preparation processes (e.g., vibrations from the nearby airfield). Equipment was deemed appropriate and functional. A system of annual performance checks for equipment was developed. Full-time analytical personnel were hired in 2017 and 2018; their first priority was the development of a Standard Operating Procedure (SOP). The SOP codified the methods used in sample preparation. Once finalized, all personnel were trained and tested in specific SOP modules. Following initial competency certification, annual proficiency exams were implemented for the analysts.

Preparation methods used for osseous remains were adapted from ones developed and validated by the Stable Isotope Preparation Laboratory at California State University, Chico, an external provider for the laboratory since 2012. The methods were validated again at the laboratory to ensure comparability of measured isotope delta values, with 50 bone samples halved and then prepared separately at each location.<sup>1</sup> Replicability (i.e., samples having the same test results given multiple preparations over time) was successfully verified using six samples of human bone and six samples of animal bone that were subsampled, then prepared in triplicate over a four-month period.

Finally, monitoring activities were developed for quality management of sample preparation. Animal bones were identified that could be prepared alongside casework samples following the “Identical Treatment” (IT) principle.<sup>2</sup> Used as a means for monitoring consistency in isotope test results, the IT principle requires that samples and reference materials are similar in chemical nature, subject to the same preparation methods, and analyzed in the same manner. Test results for these animal bones can help analysts assess replicability; they are also useful for the detection of trends that could be indicative of detrimental changes in preparation processes. As an example, a change in acid strength that resulted in slower rates of bone demineralization was found to have no impact on measured isotopic signatures of the standards.

Most of the work was completed in-house by qualified personnel; however, a disinterested external subject matter expert was also utilized. The expert completed a review of the sample preparation modules in the SOP and an onsite “over-the-shoulder audit” (i.e., “witnessing”) prior to the final on-site assessment by ANAB. The results of the review and audit prepared the laboratory for the assessment and found no discrepancies between the validated methods and the expected outcome. After all work was completed, the DPAA applied for, and was granted, accreditation of isotope sample preparation in November 2019.

### Reference(s):

1. Chesson, L.A., A.J. Edwards, E.J. Bartelink, T.H. Chau, G.E. Berg. 2020. Assessing Isotope Data Comparability: An Example from the Application of Isotope Testing to Unidentified Human Remains from Past Conflicts. *Proceedings of the American Academy of Forensic Sciences, 72<sup>nd</sup> Annual Scientific Meeting*, Anaheim, CA. 2020.
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### Method Validation, Isotope Analysis, Quality Assurance (QA)

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## A56 Choose Your Own (Accreditation) Adventure in the Defense POW/MIA Accounting Agency (DPAA) Isotope Testing Program: Part II—Sample Analysis

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**Learning Overview:** After attending this presentation, attendees will learn about the work completed to accredit isotope sample analysis under ISO/IEC 17025:2017 for Geographic Profiling (i.e., isotope testing) of human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the process used by the Defense POW/MIA Accounting Agency (DPAA) Laboratory to prepare for accreditation for isotope testing—specifically sample analysis—of unidentified individuals.

In 2017, the Defense POW/MIA Accounting Agency (DPAA) Laboratory in Hawaii began developing a series of forensic laboratory tests (“isotope testing”) for determining the “geographic profile” of unidentified remains. This is possible because the isotopic signatures of an individual’s diet and drinking water are recorded in their tissues—bone, teeth, hair, nail, etc. Preparation of tissue samples for isotope testing was accredited in 2019; another presentation describes that completed work program. This study presents the work completed in 2020 to accredit isotope sample analysis. It primarily focused on the measurement of bone collagen via Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) for nitrogen, carbon, and sulfur.

The initial step was to procure light isotope analytical instrumentation, which was ordered in January 2019 and included an EA Isolink™ attached to a DELTA V™ Plus Mass Spectrometer via a ConFlo IV™ interface (all from Thermo Fisher Scientific™). The instrumentation was officially accepted in October 2019, after all specifications preset by Thermo Fisher Scientific™ were met by the field service engineer. Certified reference materials for normalization (scaling) and quality control were also ordered from the United States Geological Survey’s Reston Stable Isotope Laboratory, the International Atomic Energy Agency, and Elemental Microanalysis. Collagen powders available as commercial products were purchased to serve as in-house standards.

Following instrument installation, a triple-gas EA-IRMS method to measure collagen was developed in-house, based on recommendations from Sayle et al.<sup>1</sup> The method was validated following a ten-point plan recommended by the Forensic Isotope Ratio Mass Spectrometry Network.<sup>2</sup> The isotopic ranges of the method are governed by available scaling reference materials and cover the expected variation of isotope delta ( $\delta$ ) values for human bone collagen: +4 to +15‰ for  $\delta^{15}\text{N}$  values, -23 to -9‰ for  $\delta^{13}\text{C}$  values, and -3 to +14‰ for  $\delta^{34}\text{S}$  values. Sample mass limits were determined by analyzing varying masses of the in-house collagen standards, ranging from 0.7mg to 1.3mg. Based on the variability of measurement results, the ideal mass range was determined to be 1.0mg  $\pm$  5%. The carryover, or memory, of the method was determined by analysis of blanks between reference materials, quality control materials, and collagen standards; no carryover was observed for the ideal mass range.

Next, precision, accuracy, and uncertainty of the method were determined. To assess precision, 18 replicates of three in-house collagen standards were analyzed seven times over five months, varying analysts and instrument operating conditions. Accuracy of the method was determined using quality control reference materials that were measured as “unknown” samples 16 times with the measured  $\delta$  value for a material compared to its certified value. Precision and accuracy data were used to calculate uncertainty, using the square root of the sum of squares rule and a coverage factor of 2 to approximate a 95% confidence interval.<sup>3</sup> Uncertainty was determined to be 0.34‰ for  $\delta^{15}\text{N}$  values, 0.30‰ for  $\delta^{13}\text{C}$  values, and 1.3‰ for  $\delta^{34}\text{S}$  values.

The EA-IRMS method was externally validated by re-analyzing 60 collagen samples previously tested by an external provider for carbon and nitrogen only. The mean difference ( $\pm$  standard deviation) between measurement results from the external provider and the DPAA Laboratory was  $-0.13 \pm 0.06\%$  for  $\delta^{15}\text{N}$  values and  $+0.19 \pm 0.08\%$  for  $\delta^{13}\text{C}$  values; both mean differences were below calculated uncertainty. Data provided by the external provider were also used to validate a Microsoft® Excel® workbook developed in-house for data processing.

Finally, the DPAA Laboratory’s Standard Operating Procedure (SOP) for isotope testing, which was initially developed during accreditation of sample preparation, was updated to encompass sample analysis. Personnel were competency certified and annual proficiency exams were implemented. Copies of the method validation report, updated SOP, and training records were provided to American National Standards Institute National Accreditation Board (ANAB). An assessment by ANAB is scheduled for November 2020, and accreditation is anticipated for 2021.

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1. Sayle K.L, C.R. Brodie, G.T. Cook, W.D. Hamilton. 2019. Sequential measurement of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values in archaeological bone collagen at the Scottish Universities Environmental Research Centre (SUERC): A new analytical frontier. *Rapid Communications in Mass Spectrometry* 33:1258-1266.
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### Method Validation, Isotope Analysis, Quality Assurance (QA)

## A57 Validating Isoscaping Methods: A Study of Oxygen, Strontium, and Sulfur

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**Learning Overview:** This presentation aims to inform attendees of the benefits and limitations of using isotopes as a means of geolocation or residency prior to death in a modern forensic setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ensuring attendees have a better understanding of this tool, leading to an improvement of the performance of isoscaping in a modern human population.

Isotopic analysis can be a useful tool for forensic anthropologists when trying to identify unknown persons. Specifically, oxygen, strontium, carbon, and nitrogen from teeth, bone, and hair have been used to evaluate and predict geographic location and diet of human remains. By using ten individuals from the Texas State Donated Skeletal Collection with known residential histories, this study aims to provide insight on the precision and accuracy of this tool. Two samples were taken from each donor, one tooth and one portion of cortical bone from the rib, in order to evaluate childhood and adult geolocations. Oxygen and strontium analyses were completed for each sample; sulfur analysis was completed only for cortical bone samples.

Oxygen and strontium analyses were prepared for each of the 20 samples and the resulting values used to create a predictive isoscape. This map was then compared to the self-reported place of birth and end-of-life residency locations. Analyses of oxygen data have been delayed due to the COVID-19 pandemic and will be available at the time of presentation. Strontium ratios accurately predicted the location of 60% of the analyzed individuals; 15% were within a 5-mile radius of the closest predicted location and the remaining 25% were within a 30-mile radius of the nearest predicted location. This research concludes that isoscapes are an effective tool but with a broad prediction range. A multi-isotope approach should be used for more precise analysis, and caution should be taken to prevent the search from becoming too narrow. Researchers should account for modern issues such as commuting and water sourcing when creating predictive isoscapes.

Sulfur analysis was also performed on each cortical bone sample to determine if sulfur isotope ratios of human bone may be useful for geolocation purposes. The ten donors were separated into inland and coastal populations, and a *T*-test was performed for assessment of regional difference and was not found to be significant ( $t(8)=2.3060, p=0.5275$ ). A visual representation was also created, but there is no visual separation between coastal and inland populations. For dietary analysis, a Pearson correlation test was performed to compare sulfur and carbon, sulfur and nitrogen, and finally carbon and nitrogen. Sulfur and carbon ( $r=0.0635, p=0.8616$ ) and sulfur and nitrogen ( $r=0.1528, p=0.6735$ ) were not found to be significantly correlated. Carbon and nitrogen ( $r=0.6698, p=0.0341$ ) were found to be of significant relation. This research concludes that sulfur analysis of bone collagen within a modern human population is not a useful geolocation tool.

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### Isotopes, Isoscaping, Identification



## A58 Mass Grave Localization Prediction With Geographical Information Systems in Guatemala and Future Impacts

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the application of easily available cross-disciplinary techniques involving data collection, geographic information systems, and statistical analyses. These techniques are combined to help narrow search areas for potential mass graves to enable the process of recovery and reunification of remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring and presenting how open-source programs and known data can be used to help predict search locations of mass graves. This investigation focuses on known data from previous exhumations in Guatemala and the use of open-source geographical information systems and data to pave the way for further research on and uses of this approach.

Conducting physical searches for mass grave locations based on anecdotal evidence is a time-consuming and resource-intensive endeavor in circumstances that often pose a threat to personal safety. The development of tools and procedures to speed such searches can greatly reduce the risk involved, increase the number of individuals whose remains are recovered and identified, and, more importantly, reunite these remains with their loved ones to provide them with a proper burial. Open-source Geographic Information Systems (GIS) software, which can analyze and manipulate the spatial characteristics of known mass grave data, represents a powerful tool that can be used to predict new mass grave locations and increase the speed and efficiency with which they are investigated.

This presentation will discuss the use of the open-source Quantum Geographic Information Systems (QGIS) project, existing mass grave locations in Guatemala from their internal armed conflict (1960s–1980s), and statistical analysis. The studied patterns were based on distance from the mass graves and change in elevation relative to roads, streets, waterways, points of interest, and possible villages/towns. Additionally, the statistical analyses performed were able to detect relationships among the variables that resulted in patterns that warrant further study and can be used to further narrow areas of investigation. The research conducted with available data in Guatemala yielded the prediction of 69% of known mass graves used for the prediction, and the search area was narrowed by eliminating 90% of the country's landmass. The patterns that provided the most predictive variables were distance of roads from mass graves, water ways, and points of interest. The methodology used in this research could provide human rights researchers, criminal investigators, and families seeking justice a powerful new set of tools, which can be deployed from the safety of a workstation.

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**Mass Graves, Open Source, Anthropology**

## A59 The Importance of Hand-Drawn Maps in Forensic Archaeological Recoveries

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**Learning Overview:** The goal of this presentation is to demonstrate the importance of forensic archaeological techniques in outdoor crime scene reconstruction, specifically in the detailed documentation of context and association via a detailed hand-drawn map.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing the importance of the hand-drawn map in forensic anthropology. Attendees will understand how hand-drawn maps are used to document context and association of evidence, remove non-forensically significant information, and simplify the presentation of evidence for the courtroom and report.

Archaeology and forensic crime scene reconstruction share a similar goal of obtaining knowledge to reconstruct and understand past events. According to Dirkmaat and Adovasio, “archaeology and crime scene reconstruction, in practice as well as in theory, share the common goals of systematically documenting, collecting, and interpreting physical evidence for the purpose of understanding the factors that affected the depositional history of the evidence.”<sup>1</sup> Thus, it is no surprise that archaeological methods are particularly useful in the reconstruction of outdoor crime scenes, with numerous authors sharing the view that effective and efficient protocols drawn from archaeology represent the best option to process outdoor crime scenes.

In the application of forensic archaeology, the establishment of context and association for all the elements in the crime scene is crucial. One of the most useful and straightforward tools toward this goal is the humble but extremely informative hand drawn map.<sup>1</sup> According to Dirkmaat et al., carefully implemented mapping procedures often reveal previously unnoticed patterns of spatial distribution of evidence and significant information about the events transpired at the crime scene.<sup>1</sup>

Properly produced hand-drawn maps will display the precise relative positions and orientation of each evidentiary item as found *in situ* at the scene. These hand-drawn maps serve different goals: (1) providing precise measurements of the placement of evidence at the scene that allow the analysis of contextual information and association patterns; (2) doing so *in situ* and in real time, as scene processing progresses, allowing immediate detection and correction of potential documentation mistakes and inconsistencies; (3) simplify the presentation of the relevant information in reports and court presentations by focusing the display only on the forensically relevant elements of the denuded scene without the distraction of non-relevant elements.<sup>1</sup>

For these purposes, hand-drawn maps are also edited and stylized to allow for easy presentation in court and reports, while still keeping their original documenting rigor and high level of detail for all relevant evidentiary information. Photographs and 3D scans cannot efficiently replace hand-drawn maps in this sense, as they do not exclude the confounding non-evidentiary information, which may hide or obscure the forensically significant patterns and associations.

This contribution presents a collection of hand-drawn maps, from actual case scenes, that highlight and illustrate different elements, issues, and techniques that have shown to be particularly useful in the casework processed by Mercyhurst University in the past three decades. The goal of this presentation is sharing some of these techniques, as well as discussing some of the most common challenges and difficulties encountered in the production of hand-drawn maps.

### Reference(s):

<sup>1</sup> Dirkmaat, Dennis C. *A Companion to Forensic Anthropology*. Chichester, West Sussex, UK: Wiley-Blackwell, 2012.

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### Forensic Archaeology, Forensic Anthropology, Crime Scene Documentation

## A60 Shining a Light on Forensic Anthropology: The Use of Alternative Light Sources (ALS) to Detect Skeletal Remains Underwater

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**Learning Overview:** The goal of this presentation, consisting of two pilot studies, is to demonstrate how ALS can be used to identify skeletal remains during terrestrial and underwater forensic investigations, explore the taphonomic impact of the aquatic environment on submerged bone, and discuss how maceration can be used as an effective preparation technique for bone undergoing laboratory ALS analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a novel use of ALS technology: to detect skeletal remains underwater, analyzed using an innovative research methodology combining custom-built fluorescence measuring software with Thermogravimetric Analysis (TGA) collagen quantification. This presentation will also introduce original complementary research into how bone collagen and consequent fluorescence levels are affected by the aquatic taphonomic environment and common maceration techniques, as well as discuss how ALS could also be used to distinguish bone from other material during excavations and crime scene investigations. This would be particularly helpful to crime scene practitioners, police divers, and search and rescue personnel.

Aquatic searches for human remains are time consuming and expensive, with current methods focusing on finding intact cadavers rather than skeletal material. ALS are a crime scene staple and are non-destructive and cost-effective, utilizing the natural autofluorescence properties of proteins to reveal latent evidence. Bone also produces fluorescence when exposed to ALS, due to a high organic content consisting primarily of collagen, providing a potential new approach to underwater searches. This research, consisting of two pilot studies, aims to demonstrate how ALS can be used to identify skeletal remains during terrestrial and underwater forensic investigations, explore the taphonomic impact of the aquatic environment on submerged bone, and discuss how maceration can be used as an effective preparation technique for bone undergoing laboratory ALS analysis.

The initial pilot study consisted of two phases, terrestrial and underwater, and used a combined methodology of digital photography to document fluorescence, bespoke C++ software to conduct fluorescence quantification, and TGA to quantify collagen. Phase 1 investigating terrestrial fluorescence identified a significant relationship between bone collagen per 1mg and observed fluorescence with an orange filter ( $R=0.260$ ,  $P=0.033$ ) when photographing porcine bone in air ( $n=17$ ), with eight organic and inorganic controls showing possible differentiation between bone and non-bone material. Phase 2 examined the impact of three aquatic conditions—canal, sea and freshwater ( $n=15$ )—on porcine bone over 21 days, including monitoring of the water chemistry; pH, turbidity, conductivity and Total Dissolved Solids (TDS). Results identified water salinity as potentially contributing to collagen loss (interval and collagen loss in sea water [ $R=0.962$ ,  $P=0.019$ ], canal water [ $R=0.925$ ,  $P=0.038$ ]), highlighting the need for further research. However, residual tissue from manual defleshing hindered fluorescence results. Consequently, a second pilot study was completed, focusing on determining the most effective method to macerate porcine bone in order to optimize fluorescence output without compromising the collagen content. Using the same photographic and quantification methodology, three maceration techniques—hot water (80°C), biological washing powder (55°C), and enzymatic (55°C)—were studied. It was found that hot water maceration proved the most successful, providing consistent fluorescence results with little impact on collagen, whereas biological washing powder was destructive to bone appearance and collagen levels. This research yielded promising results, highlighting avenues for future research, and demonstrating practical applications for missing persons cases, mass disaster investigation, and archaeological contexts. Overall, ALS examination has the potential to help simplify search methodologies, reduce costs, speed up recovery, and triage findings by aiding investigators unfamiliar with osteology to locate, differentiate, and identify possible skeletal remains.

### Bone, Fluorescence, Underwater

## A61 Variation in Bone Shrinkage Due to Thermal Exposure: A Patellar Case Study

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**Learning Overview:** After attending this presentation, attendees will see an example of thermal alteration within the same individual that is significantly higher than what is currently reported in the literature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need to expand research conducted on the percentage of bone shrinkage from thermal exposure in humans.

Most literature on bone shrinkage from thermal alteration are experimental studies utilizing animal proxies, which suggests bone may shrink upward of 20% when burned. Current literature using human bone suggests much less shrinkage, with most studies finding less than a 5% decrease in original size.<sup>1-3</sup>

This research presents a case study from a multiple individual (>1 Minimum Number of Individuals [MNI]) context involving burning. In this case, the patellae, later confirmed to be from the same individual, showed a high degree of bone shrinkage due to thermal exposure. Gross observation suggested that the shrinkage was considerably higher than the 5% documented in the literature for human bone, which initially led the analyst to consider an MNI of two rather than one. Specifically, this study analyzes differential burning between the left and right patella confirmed to be from the same individual to assess the degree of bone shrinkage in human bone and to illustrate the need to expand the research in this area.

Two observers recorded seven measurements from the posterior side of the left and right patellae: the maximum articulation width and height, width of the medial and lateral facet, volume of the entire articulation, volume of the medial and lateral facet. Measurement and observer error were assessed due to the small size of the bone and to ensure that deviations in size reflected actual bone shrinkage rather than error. Each of the measurements were collected three times per observer using Image J. The Technical Error of Measurement (TEM) was calculated for intra-observer error to verify the accuracy of repeated measurements. Absolute TEM was calculated, then transformed to relative TEM in order to obtain the error expressed as a percentage. Deviation percentages of less than 5% were considered acceptable.

The Relative TEM was under 5% for all seven measurements, with most under 2% for both observers and both patellae, suggesting low intra-observer error in measurements. The percent decrease in size (i.e., difference between patellae) was calculated using the difference in the average of the individual observer's three recordings for each of the seven measurements (left minus right side) divided by the larger size (left) for comparison. After calculating the percent decrease for the left and right patella, three measurements were consistent among both observers (<5% variation) and therefore retained for analysis of bone shrinkage: maximum articulation width and height and volume of the entire articulation.

The results show a high percentage of bone shrinkage in the right patella which was calcined (i.e., longer duration and/or higher temperature) compared to the left patella, which was only charred (i.e., less duration and/or lower temperature). The right patellar surfaces were considerably smaller than the left side from the same individual: maximum articular width was 21.7% smaller, maximum articular height 18.0% smaller, and volume of the entire articulation was 34.7% smaller. These results show a ~20% decrease in 2D metrics and a >30% decrease in volume metrics.

This case study shows that, within the same individual, thermal alteration can produce bone shrinkage that is significantly higher than what has been previously reported in the literature (5% versus 30%). This is important to consider when sorting and determining MNI in commingled contexts.

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### Thermal Alteration, Bone Shrinkage, Patella



## A62 The Implications of Thermal Alteration on Osteometry

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**Learning Overview:** After attending this presentation, attendees will understand how different thermal alterations and bone conditions affect the magnitude of changes observed on bone and how this affects an anthropologist's ability to accurately collect measurements.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to knowledge on the impact of thermal alteration on osteometric methods that are used in biological profile estimates.

Standard Operating Procedures (SOPs) in forensic anthropological analysis dictate that thermally altered remains should not be measured, which can hinder the creation of a biological profile. In South Africa, the majority of burned cases received at the Forensic Anthropology Research Centre (FARC) at the University of Pretoria, present with limited burning, which calls into question our ability to accurately measure this material and to develop biological profile parameters. Few studies have addressed estimating biological parameters from burnt remains, with the greatest focus of the existing research on cremated remains.<sup>1,2</sup> However, cremation is rarely seen in the South African forensic context compared to other burn characteristics, while limited calcination, less severe charring, and heat-altered borders are observed more commonly. The condition of the bone and the duration of the fire have been shown to have the greatest effect on the degree of thermal alteration, particularly on the degree of bone shrinkage.<sup>3</sup> Therefore, the degree of shrinkage needed to be tested on fresh and dry bone exposed to different fire durations to gauge the degree of shrinkage in the various conditions and the subsequent effect on standard anthropological measurements.

Ten standard femoral measurements were collected from 96 pig femora separated into fresh and dry categories. The fresh bones were void of flesh, but still greasy with the periosteum present, and the dry bones had some organic content lost, but had residual periosteum. Equal samples within each category were exposed to different durations of burning (5, 10, and 20 minutes). The measurements were repeated after exposure to fire. Technical Error of Measurement (TEM), Wilcoxon signed-rank, and Kruskal-Wallis tests were used to assess changes in the femoral dimensions before and after burning.

With the fresh bones, nearly all measurements collected after burning were significantly different, decreasing in size by up to 7.78% in the longest duration category. The greatest differences were observed with the maximum and bicondylar lengths as well as the lengths of the condyles. While the medial condyle length was not significantly different, the absolute TEM and relative TEM (%TEM) were 3.86mm and 5.70%, respectively. With the dry bones, all measurements collected after exposure were significantly different, decreasing in size by up to 3.98%. However, the maximum and bicondylar lengths and subtrochanteric diameters were noted to increase in size after exposure to fire. The biggest TEM and %TEM discrepancies with the dry bones were that of the medial condyle length with values of 1.70mm and 2.65%, respectively. With an increase in duration of fire exposure, there was an increase in bone fragility.

The magnitude of post-burning measurement changes was smaller for both burn conditions than has previously been reported for observer measurement errors of commonly used variables investigated for standard osteometric studies. Osteometrics on burned remains may be feasible to use in multivariate models to estimate parameters of the biological profile. Further research is needed on how the condition and duration will affect the pre- and post-burning measurements of bones other than the femur. While animal proxies are useful, there are differences in the bone compositions and, therefore, the effect of the condition of human bones and fire duration also needs to be tested.

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### Fire, Osteometry, Technical Error of Measurement

## A63 Burned Bodies: Positional Change in Decomposed and Fresh Human Remains

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**Learning Overview:** The goal of this presentation is to provide experimental data assessing body position of decomposed and fresh human remains after burning.

**Impact on the Forensic Science Community:** This presentation will positively impact the forensic science community by improving the attendee's ability to recognize pre-burn conditions of human remains through experimental examples and analysis of decomposed and fresh bodies and their behavior when burned. Thus, practitioners will be provided a reference to assist in determining whether burned remains were decomposed prior to thermal alteration.

This study examines how decomposed and fresh bodies change position when burned. Previous research indicates fresh bodies shift into a pugilistic posture during burning, but no research has been conducted to determine if decomposed remains respond differently than fresh remains.<sup>1,2</sup> The anticipated results were that there would be less positional change in the decomposed individual due to the breakdown and dehydration of tissues from decomposition processes. To test this hypothesis, a study was conducted as part of instructional courses for fire investigators at the Forensic Anthropology Center at Texas State (FACTS) Research Facility. Two donors from FACTS' whole body donation program who consented prior to death to be utilized in traumatic research were subjected to thermal trauma. One donor had decomposed on the surface for two months, had a Total Body Score (TBS) of 24, was in the process of actively desiccating, but still retained skin, muscle, and ligament tissues.<sup>3</sup> The other donor exhibited no decompositional change (TBS=3). Both were placed in a supine position into a pit on a stack of wooden pallets and were burned without the use of chemical accelerant for approximately 45–60 minutes. The event was video recorded and detailed photos of the pit, body, and pallets were taken before and after the burn for both individuals. These photos were then used to create scaled and georeferenced 3D mesh models using photogrammetric software. Using these models, coordinates for specific body locations were determined and exported with the orthoimage to Geographic Information Software (GIS). In order to examine the change of the bodies' positions without the additional variable of the pallets burning, the body positions were assessed with the pallet elevation removed.

The results indicated that both fresh and decomposed remains demonstrated movement consistent with a shift into pugilistic posture. However, the decomposed remains demonstrated a higher degree of flexion, and individual skeletal elements demonstrated a higher degree of displacement, resulting in more movement in the decomposed remains than the fresh individual. The timing of each flexion event correlated with exposure to heat and took place more rapidly in the decomposed remains. In addition, the decomposed remains experienced a complete loss of soft tissue while the fresh remains experienced only a partial loss over approximately the same length of time, resulting in more calcined bone in the decomposed individual. Factors that may have contributed to this result include less body mass, differential decomposition due to contact with the ground during decomposition, heat sensor wires interfering with flexion, and joint-driven flexion versus flexion driven by the stronger muscle flexor.

The difference in body position change between a decomposed and fresh body when burned could have important implications for forensic analysis of burned scenes. In this case, the decomposed individual demonstrated more change in body position than the fresh individual.

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### Fire, Decomposition, Body Position

## A64 Mattress Fires and Their Effect on the Destruction and Distribution of Remains in a House Fire Setting

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**Learning Overview:** After attending this presentation, attendees will understand the principles of fire behavior and the cremation process, the incidence and risk associated with mattress fires, and how, during a house fire setting, mattresses affect the destruction and distribution of remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of how mattress fires can contribute to the destruction of a body, how they affect the dispersal and recovery of cremated remains, and how this information can aid in the event reconstruction leading up to and during the fire.

Forensically, burned and cremated remains are commonly encountered, due to both the relatively high fire death rate in the United States and the continued belief that human remains can be rendered unidentifiable or destroyed through burning.<sup>1</sup> In some cases, it is necessary to recover and identify remains from residential fires, with the goal being to identify the deceased and to determine the sequence of events leading up to and during the fire.

With mattress fires being responsible for 83% of fires started in the bedroom, and the low survival odds of persons in the vicinity of an ignited mattress, this research addresses the very specific scenario of fire fatalities that involve bed-settings with regard to if and how they contribute to the enhanced destruction and displacement of a body.<sup>2</sup> It was hypothesized that those bodies located on a mattress, compared to those located on the floor nearby, would demonstrate greater degrees of cremation based on skeletal element exposure, color change, and fracture incidence. It was also hypothesized that a mattress would result in greater displacement or dispersal of remains in cases where the fire resulted in multi-story structural collapse and would limit displacement or dispersal of remains where there was little to no collapse.

To address these hypotheses, two experiments were conducted. The first experiment investigated the contribution of a mattress alone to the cremation of a body. This involved a controlled setting involving two room-sized burn cells, each containing a complete bed set with a pig (*Sus domestica*) resting on top, and another pig resting on the floor nearby. Using a small amount of accelerant, the settings were ignited and allowed to burn until extinguishment became necessary. It was discovered that while nearly all of the mattress and bedding materials were consumed, all the carcasses remained largely intact. It was concluded, therefore, that while mattresses are considered a significant fuel source within the home, they alone do not significantly contribute to the destruction of a body to a point that would hinder identification or recovery.<sup>3</sup>

The second experiment investigated how a mattress affects both the destruction and dispersal of remains in a residential fire setting. Within a small single-story home, eight pigs were distributed throughout the floorplan—five on mattresses, three on nearby floors. The house was then ignited and allowed to burn without any extinguishment efforts. Subject remains were then mapped, recovered, and evaluated for cremation state. It was discovered that mattress specimens showed advanced cremation-related decomposition and fracturing when compared to the floor counterparts. It was also confirmed that mattresses have a variable effect on remains distribution dependent on the distance they fall during structural collapse. Those falling short distances showed limited, if any, dispersal, while those falling large distances were more greatly dispersed as a result of impact and interfering objects. Based on this research, investigators may be able to reconstruct a victim's final location and moments.

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### Forensic Anthropology, Mattress Fires, Cremation

## A65 Pack Rats: An Unconsidered Taphonomic Variable

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**Learning Overview:** After attending this presentation, attendees will possess an understanding of how pack rats have been underestimated as a variable in forensic taphonomy and their potential influence to a forensic scene and scene recovery efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding of how pack rats can alter a scene. Additionally, guidance on pack rat nest identification and the need to excavate nests to find human remains and other potential evidence is provided.

Forensic taphonomy is a broad discipline that studies many variables that may influence the deposition of skeletal remains in modern contexts. It is well documented that animal activity, such as carnivore scavenging, will scatter and destroy remains, resulting in the loss and reduction of elements recovered from a forensic scene. Pack rats as a taphonomic variable have been discussed in the archaeological and paleontological literature; however, there is limited discussion of pack rats in the forensic literature. Although rodent modification to a bone's surface is a common finding, the thought of a rodent as possibly the primary actor in scattering remains is not.

This presentation provides four case examples from forensic scene recoveries in Oklahoma where pack rats were one of the primary taphonomic variables resulting in the postmortem movement and caching of skeletal elements in nests. The number of pack rat nests excavated ranged from 1 up to 17. Nest construction varied from free standing, built into shrubbery, or located adjacent to the burrows of other animals. The nests were excavated by hand and with hand tools, such as bow rakes, to carefully disassemble the twigs and branches to find the skeletal elements entwined within. Images of differently sized nests are displayed to demonstrate variation in their size and construction style. Distances from the primary scatter location to the nests ranged from 13 meters up to 62 meters in the examples discussed. The number of elements recovered from a single nest ranged from 3 up to 27 elements, along with multiple fragments of clothing. Size of the skeletal material moved by the pack rats included small hand and foot bones up to elements as large as a sacrum and fibula. The amount of chewing modification to the elements varied from minimal to severe. A map displaying the range of pack rats will be displayed to highlight the regions in the United States where this rodent may play a role in the dispersal and caching of skeletal elements and other potential evidentiary items.

Understanding the role pack rats may play in the dispersal and hording of skeletal elements is a benefit to all parties involved in forensic scene recovery efforts. Identifying pack rat nests and the need to excavate them will help maximize the recovery of skeletal elements and potentially aid in identification and trauma analysis of skeletal remains.

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### Forensic Taphonomy, Pack Rat Nests, Scene Recovery

## A66 Phase II Spatial Patterning of Vulture-Scavenged Human Remains

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**Learning Overview:** After attending this presentation, attendees will better understand vulture-scavenging patterns of human remains at Texas State University's Forensic Anthropology Research Facility (FARF).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that vultures' scavenging activities can affect Postmortem Interval (PMI) estimation as well as provide longitudinal data that contributes to the development of vulture-modified decomposition sequences.

This project is Phase II of a longitudinal study.<sup>1</sup> Phase II continues to systematically assess the timing and sequence, rate of skeletonization, disarticulation, and dispersal of human remains by vulture-scavenging activity. Additionally, it investigates whether microenvironment (e.g., placement of the bodies in proximity to vegetation or other human remains) affect vulture-scavenging activity. While some studies have examined scavenger guild behavior, none have modeled the ways microenvironmental factors might influence vulture scavenging of human remains.<sup>2</sup>

**Data and Methods:** Five donated human bodies from the Willed Body Donation Program at Texas State University were placed at the University's FARF in various contexts. For example, placements were chosen to reflect an individual in heat distress seeking shade or multiple individuals perishing in close proximity to each other. The FARF is a closed, 16-acre site (reinforced chain-linked fence), which allows for exclusive observation of avian scavengers. The first donated body (Body 1) was placed at FARF on September 3, 2013, in a grassy area surrounded by trees. Body 2 was placed on September 24, 2013, in open grassland. Body 3 was placed on November 21, 2013, adjacent to a wooded area approximately 66ft west of Body 1. Body 4 was placed approximately 364ft west from all the other bodies in open grassland on December 11, 2013. Last, Body 5 was placed on May 2, 2014, in open grassland, adjacent to a tree cluster, approximately 66ft north of Body 2.

Accumulated Degree Days (ADD) (i.e., the energy available to drive biological processes) was calculated to characterize the geographic region and explain the level of observed decomposition before complete skeletonization by vultures.<sup>3</sup> Animal activity was captured with motion-sensing infrared game cameras (Moultrie Game Spy<sup>®</sup>) and high-accuracy Global Positioning System (GPS) (GeoExplorer XT<sup>®</sup>) was used to systematically record successive disarticulation and scattering. GPS data were input into a Geographic Information Systems (GIS) to calculate elevation, Mean Center (MC), Standard Distance (SD), and Directional Distribution (DD) changes to analyze and visualize vulture-scavenging activity patterns.

**Results and Conclusions:** Phase II results align the findings from the Phase I.<sup>1</sup> Specifically, all remains—with the exception of Body 4—were moved from higher to lower elevations, vultures were observed continuing to disperse body parts after skeletonization, and vultures can completely skeletonize a set of human remains within 72 hours once scavenging begins. Notably, vultures began to scavenge at variable times after placement (anywhere from 4 to 77 days). Further, the data collected inform general considerations regarding the effect of microenvironment, such as the difference in Time-to-Scavenging (TtS).

TtS may be related to temperature, not tree cover or proximity. Vultures appeared to wait for shorter amounts of time to begin scavenging when the bodies were placed in the autumn (Body 1 and Body 2) or summer (Body 5) compared to winter (Body 3 and Body 4). It is possible that in Central Texas the initiation of scavenging by vultures is shorter in the warm to hot months. Temperature may also affect the number of vultures that will scavenge at a given time. Larger numbers of vultures may scavenge together in cooler months (Body 2 and Body 3), while smaller groups may scavenge together more frequently in hotter months (Body 5).

Taken together, Phases I and II demonstrate that vulture-scavenging activity can impact the estimation of PMI, in this case increasing the estimate, and introduce additional challenges to recovering remains by authorities.

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### Vulture Scavenging, Postmortem Interval Estimation, Geographic Information Systems

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## A67 Vulture and Black-Backed Jackal Scavenging: Forensic Implications for the Recovery of Scattered Remains in South Africa

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**Learning Overview:** The goals of this presentation are to present the scattering patterns of vultures and black-backed jackals in South Africa and to highlight the influence of specific environmental features on the scattering patterns. The principles of the link search method, and their combination with the grid search pattern, are outlined as the ideal search method for scattered remains.

**Impact on the Forensic Science Community:** Scavenging animals occur globally. This presentation will impact the forensic science community by providing a better understanding of scavenger scattering patterns that will assist forensic specialists in the holistic recovery of scattered remains. The basic recovery principles of the link search method, combined with grid search pattern, are applicable internationally.

Twenty pig carcasses (*Sus scrofa domesticus*) (40–80kg) were placed at two research sites (ten at each site). Each site represented a different environment in South Africa. The Wits Rural Facility in the Limpopo province (Lowveld) represents a rural wildlife environment. The Mierjie Le Roux Experimental Farm in the Gauteng province (Highveld) represents a peri-urban agricultural environment. Vultures (white-backed, lappet-faced, and hooded vultures) were the dominant scavengers in the Lowveld site and black-backed jackals were dominant in the Highveld site.

Vulture scavenging resulted in the rapid skeletonization of the pig carcasses between 5 and 98 minutes. The skeletal remains were scattered within a circular area of 157.9m<sup>2</sup>/1705.5ft<sup>2</sup>, with a radius of 7.09m/23.3ft. Black-backed jackal scavenging resulted in the skeletonization of the pig carcasses between 1 and 42 days. The skeletal remains were scattered linearly, in two general directions within a 90° arc, over a maximum distance of 73.7m/241.8ft. The scattering pattern of vultures was heavily influenced by the proximity of fences (particularly electrified fences). The pattern of black-backed jackal scattering was heavily influenced by the restriction of movement by fences, the location of their dens, and the presence of established animal trails. The link search method, in conjunction with the grid pattern method, increased the number of recovered remains. By combining the two methods, the recovery of remains scattered over a large area was improved.

This combined search method is more flexible than other methods and relies on the searcher to adjust their direction based on the identification of scattering cues. Although the scattering patterns highlighted in this study are focused on South African scavengers, scavenging occurs globally and the suggested recovery method could be successfully implemented internationally in forensic cases that have been scattered by scavenging animals.

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### Scavenging, Scattering Patterns, South Africa



## **A68 Taphonomic Alterations of Skeletal Remains Within a Subtropic Region: A Key West Cemetery**

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**WITHDRAWN**

## A69 Taphonomic Effects of Acidic Soil on Human Remains in Japan: A Preliminary Study

Brian D. Padgett, PhD\*, The Ohio State University, Columbus, OH 43214

**Learning Overview:** After attending this presentation, attendees will be more familiar with unique taphonomic features in human osseous remains that indicate prolonged exposure to acidic soil.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the effects of unusual decompositional processes that may be mistaken for non-taphonomic alterations to the analyst unfamiliar with the effects of acidic soil on human remains.

Criminal investigation methodology has a long history in Japan, though research in forensic anthropology has been somewhat limited, possibly due to historically low crime rates, a lack of forensic research facilities, and reluctance among people to donate their bodies to science, owing to various cultural concerns, though the latter issue is slowly changing.<sup>1-4</sup> Because of the current status of forensic anthropology in Japan, certain archaeological collections can provide valuable insight into the taphonomic effects that result from prolonged postmortem contact with the soil found there.

The islands of Japan are volcanically active, which has had an effect on soil composition. The soil of central and southern Japan are mainly Ultisols or Oxisols, with average pH levels of 6.5 to 4.5 and are relatively low in iron.<sup>5</sup> The low pH levels, indicating high acidity, are due mainly to the sulfurous compounds found in volcanic emissions such as ash.<sup>6</sup> Due to this high soil acidity, bones and other organic materials generally do not preserve well, unless they are deposited in such a way as to seal them off from the soil matrix and/or they are deposited in a very alkaline environment, such as shell middens.<sup>7</sup>

The archaeological collections used in this study to examine the effects of an acidic environment on human remains were all recovered from sites dated to the Yayoi period (~900 BCE to 250 CE) in either western Honshu or northern Kyushu. Yayoi burials of this region were either inhumations without a burial container, or the bodies were placed in wooden coffins, stone sarcophagi, or in large ceramic jars called *kamekan*.<sup>8-12</sup> These different styles of interment afforded varying levels of protection, which resulted in an observable gradient in preservation. There are fewer extant human remains from later periods due to a decline in the use of durable containers and a gradual tendency toward cremation as a means of disposal. Thus, Yayoi period burials of this region are optimal for study here.

Observations among recovered remains reveal that the acidic soil can have a detrimental effect on bones, but with some recognizable patterns of cortical attrition. Direct contact with the soil can result in a slow dissolution of the structure, while the portion of the structure not in contact with the soil may remain intact. If a burial container became compromised during interment, water dripping on the remains could result in highly conserved taphonomic defects. Recognition of the results of prolonged deposition in this environment is important to avoid conflating this particular postmortem alteration with a non-taphonomic etiology.

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### Taphonomy, Decomposition, East Asia



## A70 The Application and Implication of Radiocarbon Dating in Forensic Case Work: When Medicolegal Significance Meets Archaeological Relevance

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**Learning Overview:** After attending this presentation, attendees will appreciate the benefits as well as the implications of both traditional radiocarbon dating and bomb pulse dating when applied to skeletonized remains to estimate time since death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) increasing knowledge of the potential applications of radiocarbon techniques to skeletal remains routinely encountered in forensic casework, (2) discussing implementation with or without contextual information, and (3) illustrating an original scientific collaboration with French archaeological authorities.

The estimation of the time frame between death and discovery of an individual is one of the essential aspects in forensic anthropology. In France, prosecution of crimes is barred after 20 years; however, determining the forensic significance of skeletonized remains in an area of high archaeological potential can be challenging. Through two complementary cases, this communication illustrates the application of radiocarbon analysis by examining their medicolegal significance.

Due to the half-life period of radiocarbon (5,730 years), dating techniques based on this radioisotope are typically relevant to material that dates from 300 to 55,000 years. Hence, traditional radiocarbon has the potential to determine whether the remains are of archaeological or forensic significance. When remains belong to a contemporaneous time frame, artificial levels of radiocarbon due to above-ground testing of nuclear weapons in the 1950s/1960s, described as “bomb peak,” can determine if remains are forensically relevant.

In 2019, adult human remains representing a single and incomplete skeleton were accidentally discovered in a flooded underground fortification built in the late 19<sup>th</sup> century near Wimereux in Northern France. Because the fort was operational during World War I, became part of the “Atlantic Wall” during World War II, and was sporadically occupied by homeless individuals, the estimated time since death may range from a couple of years to a century. Contextual information was uncertain as a result of severe flooding and site abandonment. A sample of cortical bone from the femur was submitted to radiocarbon dating after extraction of collagen fraction on the Accelerator Mass Spectrometry (AMS) facility of Saclay. Results showed elevated radiocarbon levels associated with the bomb peak and indicated two calibrated date ranges: (1) 1962 AD (37.3%), and (2) 1974/1975 AD (58.1%). Even considering acknowledged limitations of bomb pulse dating, calibrated ages are outside the limitations of the 20-year period ruled by French criminal law.

In 2017, an incomplete but well-preserved adult skull was discovered on the beach at Audresselles in Northern France without contextual information about the postmortem interval. A sample of a parietal bone was submitted for radiocarbon measurement, and low radiocarbon levels indicated no forensic relevance. Surprisingly, the radiocarbon age was  $5,387 \pm 18$  years Before Present (BP) (cal 4,232 BC at 92.5%), indicating a high archaeological relevance. Archaeological authorities were informed to ensure appropriate procedure and to contribute to the archaeological geo-referenced database implemented by the Ministry of Culture.

Reliable distinction between archaeological and forensic remains is of crucial importance. Radiocarbon is a key tool to shed light on the forensic relevance when contextual information is lost and provide a mutual benefit for forensic anthropologists and archaeologists.

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### Radiocarbon Dating, Time Since Death, Forensic Anthropology

## A71 Investigating the Extent of Bone Diagenesis in Short Timescales Through a Histological Approach

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**Learning Overview:** After attending this presentation, attendees will understand the potential for bone diagenesis, in the form of microbial infiltration, to occur in short forensic (<28 weeks) timescales, as well as the influences microbes, such as those present within the gut microbiome and burial soil, can play. This study will present a potential way of quantifying the bioerosion occurring in bone through the novel method of counting normal osteocyte lacunae present within the sample.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing existing knowledge of microbial action on skeletal remains. This presentation will show that microbial infiltration occurs to skeletal tissue in the early postmortem period, before skeletonization has occurred, and in partial remains without the inclusion of gut bacteria, highlighting the role the deposition environment plays in bioerosion

The evaluation of bone diagenetic phenomena in archaeological timescales has a long history, yet its occurrence in short timescales has been overlooked.<sup>1,2</sup> The presence of the gut bacteria drive microbial infiltration, but the role of the deposition environment in the early postmortem period is unclear.<sup>3,4</sup> Presented here is a short timescale study that aimed to establish the extent of diagenesis observed on the internal microstructure of skeletal tissue, using the Oxford Histological Index as well as a novel method of counting the normal osteocyte lacunae present within the sample, to assess the level of microbial infiltration occurring over time.<sup>5</sup>

It was hypothesized that microbial infiltration would be seen within 28 weeks, with sample retrievals occurring every 4 weeks. Due to the sensitivity of the diagenetic processes being observed, three tissue types were used: whole remains to include gut bacteria; excised, fleshed limbs for autolytic effects and exclusion of gut bacteria; and defleshed long bones to exclude bacteria associated with soft tissue decomposition. All of these were taken from domestic rats (*Rattus rattus*) in two deposition environments: exposed on a clean plastic surface to allow insect access but exclude soil microbes; and buried in garden soil to include soil microbes. Upon retrieval, the samples were cleaned, and a manual preparation method was used to produce histological sections of around 100µm thick, allowing the lacunae within the microstructure to be observed and quantified using a light microscope.<sup>6</sup>

Results showed that microbial infiltration can occur in short timescales, with alterations to the bone being observed in the early postmortem period (< 28 weeks) in all tissue types ( $p=0.00$ ). Despite previous observations that many factors can affect bone diagenesis, few differences were found in the extent of diagenetic change when comparing the different tissue types ( $p=0.505$ ) and when comparing the two deposition environments ( $p=0.532$ ).<sup>7,8</sup>

This study showed that it is possible for microbial infiltrations to occur to the skeletal tissue in the early postmortem period (<28 weeks), before skeletonization occurs. It has also shown that microbial infiltrations can occur in partial samples without the inclusion of the gut bacteria to drive bone diagenesis, indicating that the deposition environment plays an important role in the deterioration of skeletal remains.

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### Bone Diagenesis, Histology, Bioerosion

## A72 The Growth of Bryozoan Colonies on Immersed Human Bones: How Can It Help to Estimate the Minimum Postmortem Interval?

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**Learning Overview:** After attending this presentation, attendees will have a better appreciation of anthropological cases with bones colonized by marine organisms such as bryozoans.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the potential usefulness of bryozoan colony development on human bones.

Human bones are regularly discovered in seas or oceans, and forensic institutes near coastal regions are frequently asked for an analysis. In such cases, the interpretation of lesions and the dating of skeletal remains can be delicate. Indeed, the marine environments are different from the terrestrial ones and are also very variable. Specific taphonomic changes or fauna intervention can also occur on the immersed remains. Sometimes, marine species adhere to the bone surface, and this occurrence can hinder the interpretation or, on the contrary, can be useful for some part of the anthropological examination.<sup>1</sup> However, the forensic literature is relatively limited concerning those situations.

This study reports the case of an incomplete human skull discovered in a fishing net from a trawler in the North Sea, approximately 10 kilometers away from the French coasts. The cranial vault presented characteristic lesions of a prolonged marine immersion, with a chalky surface texture and an overall bleaching and battering of the bone surface. Several bryozoan colonies were observed on different outer surfaces of the skull and especially on its lower part. Three postmortem set lesions were also present on the calvaria, associating linear abrasions of the outer table centered by penetrating holes exposing the diploe. Those lesions were compatible with an interaction with a part of a fishing net, with one net removing some of the Bryozoan colonies.

Bryozoa (phylum), also known as Ectoprocta, are aquatic and microscopic invertebrates that form colonies made from individuals called zooids of about 0.5mm in size. They are included in an organic or inorganic exoskeleton that may persist after the death of bryozoans. Their growth speed varies depending on the species, and also on local food, quality of the substrate, and physical parameters of the environment.<sup>2</sup> The bryozoan colony growth form varies, according to the pattern of addition of zooids: it starts as a single zooid and new ones are added sequentially in a regular arrangement, in a line, at the tips of branches or along an edge (of the substrate or an erect species). After identification of the bryozoan species and consulting the literature concerning their growth speed, a minimal immersion time of the bones could be determined. In the presented case, colonies have the characteristics of *Membranipora sp.*, which size corresponds to a growth time that could vary from a few months to multiple years. This estimation could be completed by adding the period of skeletonization of the remains in the marine environment and the bone surface colonization by bryozoans. However, a recent review of the literature emphasizes the lack of standardized methodology about the study of the different bryozoan species and growth forms.<sup>3</sup> The biology of such organisms is indeed complex, and a cooperation between professionals in the forensic field and marine biologists seems to be crucial in order to develop this approach, create a specific database, and participate in the estimation of the minimum postmortem interval.

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### Taphonomy, Postmortem Interval Estimation, Forensic Anthropology

## A73 An Analysis of Clothed Pig Proxies (*Sus Scrofa*) When Cold Temperatures Are Present in Central Illinois

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**Learning Overview:** After attending this presentation, attendees will better understand the decompositional changes that occur when clothed, buried pig proxies are subjected to the cold temperatures and weather conditions of central Illinois.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing preliminary insights of cold temperature effects on the decomposition processes of clothed specimens that have been placed in shallow and deep burials by using dismembered pig proxies in place of human cadavers.

Relatively little is known on how cold temperatures and associated weather conditions influence the rate and processes of decomposition on buried remains. Understanding how these factors influence taphonomic variables is important due to the presence of winter weather phenomena across the world. When ambient temperatures drop to below 37°F, decomposition is usually reduced or can be halted. Few studies have been conducted to track the quantitative and qualitative decompositional changes that occur to human and/or animal remains when cold temperatures (<37°F) and winter weather conditions (i.e., snow, sleet, ice, frost) are present over the course of several months. It was hypothesized that the freezing temperatures of central Illinois would inhibit the taphonomic process of buried pig remains, and, after a period of four months, those placed in deep burials would be in an earlier stage of decomposition than those in a shallow burial.

From early January to early May 2018, a total of four adult partial pig specimens labeled A–D were buried at depths of 6 inches (shallow burial) and 18 inches (deep burial) below the ground surface. Specimens consisted of the hind legs of domestic pigs (*Sus scrofa*), which were cut into thirds prior to burial. None of the four specimens had been frozen prior to the experiment. Each proxy was comprised of skin, fat, muscle, bone, and bone marrow and would be close in comparison to a dismembered human limb. All four specimens were wrapped individually in a single white cotton t-shirt prior to burial, with A and C placed at 18 inches and B and D placed at 6 inches below the ground surface. After taking initial photographs and measurements (i.e., circumference and weight), specimens were buried at the testing site located in Normal, IL. A thorough examination was conducted on specimens A and B after two months, and then after four months on specimens C and D. During this time, specimens were photographed, weighed, measured, and written observations on insect activity and weather data (i.e., temperature, humidity, precipitation) were gathered. Weather data were also obtained daily from the United States Weather Service for Normal, IL. Decomposition stage scoring was based on Galloway and colleagues' methodology with some modification: fresh, early decomposition, advanced decomposition, and skeletonization.<sup>1</sup>

At the two-month interval, specimen B (6 inches) appeared relatively fresh, which was in contrast to specimen A (18 inches) that appeared paler in color with the soft tissue beginning to liquefy. At the four-month interval, specimen C (18 inches) was completely liquefied with maggots on the external surface of the t-shirt. In contrast, specimen D (6 inches) had fewer maggots than specimen C as well as a less advanced stage of liquefaction in which the skin, muscle, and fat were still discernable. Specimens at 18 inches experienced more marked evidence of decomposition after two and four months compared to their counterparts buried at 6 inches. This was evident by a greater percentage loss in weight and an increase in circumference due to the continued breakdown of tissues.

Based on this study's results, which contradict previously conducted studies on burial depth, the hypothesis was rejected. Burial depth in geographic regions with prolonged cold ambient temperatures, such as central Illinois, plays a role when using clothed pig proxies. Findings suggest that when cold ambient temperatures are present, an increase in burial depth correlates to an advanced rate of decomposition. Ultimately, while the examination of buried pig proxies allows for a better understanding of real-world decomposition processes of dismembered limbs, the use of human cadavers would provide the most valuable insight of the decomposition process in these settings.

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### Forensic Taphonomy, Decomposition, Cold Temperatures

## A74 Decomposition in the Sonoran Desert During the Summer

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**Learning Overview:** The major goal of this presentation is to offer a timeline of decomposition for use in estimating postmortem interval in the southwestern United States, particularly during the summer months when decomposition is rapid.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a model for estimating postmortem interval during the summer months in the Sonoran Desert. The extreme heat and aridity of this location directly affect the mortality of exposed populations and the subsequent decomposition timing and stages. Patterns of decompositional change gleaned from other environments are not applicable to postmortem interval estimation in this extreme environment. This study is the first in a series to document the stages of decomposition in the Sonoran Desert throughout the year.

The extreme climate of the Sonoran Desert of the southwestern United States presents a unique challenge for estimating postmortem intervals through stages of decomposition. In a single summer month, temperatures were recorded ranging between 58°F and 124°F, with relative humidity as low as 7%. In the Arizonan portion of this desert, the city of Phoenix in Maricopa County has been described as a “heat island” with temperatures up to 21°F hotter than rural areas.<sup>1</sup>

Maricopa County is part of the Tucson Sector of the United States-Mexico border, where more than 200 sets of remains known to belong to undocumented border crossers have been recovered in recent years.<sup>2</sup> In addition, Phoenix also has a crisis of homelessness. Maricopa County reported a total of 25,832 homeless individuals in 2015.<sup>3</sup> Exposure during the summer is a common cause of death among these groups.<sup>4</sup> Further compounding this unique vulnerability to environmental factors is the shared likelihood for a potentially increased interval between death and the point at which a deceased individual may be recovered. As an accurate estimate of postmortem interval is a critical component of the identification of the deceased in these cases, an improved understanding of the rates and stages of decomposition in this environment is crucial.

The goals of this study were to: (1) document the process of decomposition and the effects of this extreme environment, and (2) estimate the timing and stages of decomposition during the summer in the Sonoran Desert of Maricopa County. To achieve these goals, two intact adult pig carcasses were placed in cages in an area of native desert in mid-June. Both were approximately the size of a human adult (~200lbs) and were clothed to better represent the state of individuals who perish in the desert. One carcass was placed in direct sunlight and the other was placed in the shade of a Palo Verde tree. Cameras were set to capture photos at 30-minute intervals. Additionally, weather stations were set up near each cage to collect temperature and humidity readings every ten minutes. Further photographs and documentation were collected throughout the experiment.

The results of this study demonstrate both the impact of extreme heat and aridity on the decomposition process overall, as well as the nuanced differences in the timing and stages of decomposition produced by differential ultraviolet radiation.<sup>5</sup> The carcass placed directly in the sun experienced abdominal wall rupture within 12 hours. Flies were present in large quantities within 24 hours, and maggot activity peaked after five days. Insect activity occurred predominantly at night on the underside of the body and under clothing. This carcass maintained a wet appearance, and skeletal exposure was only seen on the facial region. The carcass placed in the shade did not experience abdominal wall rupture, and the soft tissue began to mummify around regions of skeletal exposure after seven days. More extensive maggot activity was noted, as was insect activity during both day and night. Maggot activity peaked after six days. Throughout the experiment, rodent and bird activity was noted during early morning, late evening, and nighttime hours. Within one week, the carcass in the shade exhibited skeletal exposure in the face and around the vertebral elements. No such exposure was observed in the other carcass. In the subsequent weeks, no further changes were visible in either set of remains following two weeks, and complete skeletonization was not observed when the experiment was concluded after 26 days.

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### Decomposition, Postmortem Interval, Tucson Secto

## A75 Forensic Anthropology Casework Performance: Assessing Accuracy and Trends for Biological Profile Estimates on a Comprehensive Sample of Identified Decedent Cases

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**Learning Overview:** After attending this presentation, attendees will better understand the utility of the Forensic Anthropology Database for Assessing Methods Accuracy (FADAMA) data for highlighting performance trends in forensic anthropology and how it can help target future method and sampling improvement.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of FADAMA data for critically assessing performance trends in the field of forensic anthropology.

FADAMA (<https://www-app.igb.illinois.edu/sofadb/>) is an online repository for data from identified skeletal cases. The purpose of FADAMA was to establish a tool with which forensic anthropologists can ask, analyze, and answer questions of casework and method performance on a community-wide scale. Such work requires a large number of cases in which the biological profile of the decedent is confirmed. FADAMA provides the first free and virtual organized space to share approaches to casework and casework outcomes. FADAMA is sponsored and maintained by the Society of Forensic Anthropologists.

After attending this presentation, attendees will understand the accuracy rates and trends in forensic anthropology casework, specifically regarding the assessment of the biological profile. FADAMA cases ( $n=359$ ) were analyzed to explore the following: (1) overall accuracy rates per biological profile component; (2) whether trends (e.g., demographic; reporting) exist related to inaccuracy rates; and (3) overall case-level performance in assessing the biological profile.

Accuracy rates for the four biological profile components ranged from 86% to 98%, with sex estimates performing the best and stature performing the poorest, with ancestry (94%) and age in the middle (92%). Age estimations are the most frequently reported biological profile component (340 out of 359), while stature is the least frequently reported component (101 out of 359).

Analyses of trends in accuracy rates per biological profile component yielded noteworthy results. For stature, cases with inaccurate ( $n = 14$ ) estimates were more frequently a result of overestimating stature ( $n = 12$ ) than underestimating ( $n = 2$ ). Cases that overestimated stature tend to have smaller errors (0.14"–3.83") compared with those cases that underestimated (3"–6.3"). Univariate non-parametric tests revealed that identified sex, age, stature, and race did not yield a statistically significant relationship with stature accuracy. However, 8 of the 14 inaccurate stature estimates were Hispanic individuals.

For sex, the majority of the identified FADAMA cases were male (70%). While the overall sex estimation inaccuracies were the lowest of any biological profile component, females were missexed approximately ten times more often than males.

For ancestry, the majority of identified individuals were White (48%), with African American (23%) and Hispanic (23%) the next most-common categories. Regarding ancestry estimation performance, African Americans and Whites had the lowest inaccuracy rates (3.6% and 3.2%), while Hispanics (11.9%) and Asian/Pacific Islanders (22.2%) demonstrated substantial inaccuracy rates. Chi-squared tests confirmed the significance of these trends between identified race and ancestry estimation accuracy ( $n = 248$ , Pearson  $p = 0.019$ ). Furthermore, identified sex was related to ancestry estimation accuracy ( $n = 247$ , Pearson  $p = 0.047$ ), with females' ancestry more frequently inaccurately estimated.

For age estimates, inaccuracies did not occur more frequently in a particular age cohort (young/middle/older adults). For inaccurate cases, error rate was balanced between underestimating ( $n = 15$ ) and overestimating ( $n = 13$ ) identified age. However, cases that overestimated age tended to have greater errors. Reported age ranges appear to increase per age cohort, such that the older adults generally had the largest ranges (median = 39 years), while the young adults had the smallest age ranges (median = 10 years).

Regarding case-level performance trends, for the cases with submitted estimates for all four biological profile components ( $n = 78$ ), 76% accurately estimated all four, while 20.5% got one component wrong, and 4% inaccurately estimated two components. When considering all cases that submitted estimates for at least one biological profile component ( $n = 358$ ), it was found that as the number of components estimated increases, so does the number of inaccurate estimations.

This presentation impacts the forensic science community by demonstrating the utility of FADAMA data for highlighting performance trends in forensic anthropology and can help target future method and sampling improvement. As case data continue to be added to FADAMA, forensic anthropologists can gain a more comprehensive perspective on the state of casework.

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### Anthropology, Accuracy, Biological Profile

## A76 Variation in Pubic Body Shape in Hispanic and Euroamerican Populations and the Implications for Assessing Biological Sex From Skeletal Remains

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the range of human variation in the shape of the pubic bone and will be able to apply this understanding to more reliably evaluate biological sex. Additionally, attendees will gain an understanding of the use and effectiveness of Elliptical Fourier Analysis (EFA) for describing human skeletal variation. The learning objectives include: (1) identifying the range of variation in the shape of the human pubic bone, (2) demonstrating the wider range of variation in Hispanic populations and in males, (3) understanding that the vast majority of skeletal variation in morphology cannot currently be explained, and (4) demonstrating the usefulness of EFA in descriptive studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the importance of understanding the complexity of shape variation in the human skeleton.

Sex determination is a critical step in the creation of a biological profile to aid in the identification of human remains; a positive identification of skeletonized remains is often not possible until the decedent's biological traits have been evaluated accurately, narrowing the range of possible matches. One of the most reliable indicators of sex in the human skeleton is the pubic bone, a highly sexually dimorphic bone due to the constraints of childbirth in females. Current methods for sex determination based on the pubic bone depend on somewhat subjectively defined observations of complex morphological features and are thus prone to inter- and intra-observer error. Additionally, many of these methods are based on North American populations alone and therefore may not accurately model the variation that exists in other populations.

In this study, EFA was conducted using photographs of 451 pubic bones of individuals of North American Hispanic (39), Chilean (121), and Euroamerican (286) descent. Principal Components Analysis (PCA) then determined the patterns of variation in the shape of the pubic bone. Analysis of Covariance (ANCOVA) was run to determine which covariates (sex, age at death, ancestry, and the interaction between sex and ancestry) significantly affect these shape patterns. These data were analyzed to address the null hypotheses that there are no significant differences in the shape of the pubic body: (1) between males and females, (2) between Hispanics and Euroamericans, (3) between individuals of different adult ages, or (4) as a result of any interaction between sex and ancestry.

EFA generated five effective Principal Components (PCs), which together described approximately 95% of the overall variation in the shape of the pubic bone. The covariates that were found to significantly influence these shapes include sex, ancestry, and age at death; the interaction between sex and ancestry was not significant. The significant covariates explained only 25% of the overall variation in the shape of the pubic bone, with the majority of this explained by sex alone. Differences between Hispanics and Euroamericans were consistently small. The remaining 75% of shape variation that cannot be explained is likely influenced by other variables not controlled for in this study.

EFA was found to be an effective tool for describing variation but is difficult to apply in a practical setting. EFA did, however, demonstrate that the pubic bone of Hispanic populations does not substantively differ in shape from those of Euroamericans. Hispanics are significantly understudied and underrepresented in the methods currently in use in the United States. This study demonstrates that the pubic bone can be used as a reliable determinant of sex for the populations included in the sample, and that the majority of variance in skeletal morphology remains unexplained.

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### Forensic Anthropology, Human Variation, Biological Profile

## A77 Reference Sample Optimization for Juvenile Age Estimation From Diaphyseal Lengths

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**Learning Overview:** After attending this presentation, attendees will understand how to evaluate the parameters of a good reference sample in juvenile age estimation from diaphyseal lengths, including prioritizing reference sample size over specificity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by challenging the assumption that reference sample specificity should be prioritized in juvenile age estimation and demonstrating the research potential of evaluating methods using age intervals rather than point estimates alone.

Population specificity of reference samples for biological profile methods is seeing increased attention in forensic anthropology. The assumption that a more specific reference sample will produce better estimates deserves a close look in juvenile age estimation. Relative to adults, juvenile trait-age correlations are high, and sex and ancestry estimation is challenging. The project objective is to compare the importance of reference sample size and specificity when estimating age from long bone lengths. Two competing hypotheses are tested: first, whether better age estimation results will be obtained by using a more specific reference sample, and second, whether better age estimation results will be obtained by using a larger reference sample.

The data consist of diaphyseal length measurements of the humerus, radius, ulna, femur, tibia, and fibula from 102 South African girls, 203 South African boys, 52 United States girls, and 86 United States boys (age range: 1–13 years). First, model stability was measured under varying conditions of reference sample heterogeneity and size. Model stability was calculated as the pooled standard error of two univariate linear models built using reference samples of equal sizes. Reference sample size was varied from 40%–100% of the original size. Reference sample heterogeneity by country for a single sex and sex for a single country was varied from 0%–50% for each of the six long bones in a full factorial design.

To test model performance, ages were estimated using a Bayesian (length dependent on age) multivariate normal linear model with trait covariances included. With each of the four specific sex/country combinations as a test sample, the model was trained on the full sample, the opposite specific group, and the same specific group. When the same group was in the training and test samples, leave-one-out cross-validation was used so that the same individual was never in both the training and test samples for an age estimate. Success rate was measured as the proportion of individuals whose true age fell within a 95% estimated probability interval for age.

In the model stability testing, standard error increased with decreasing sample size, while heterogeneity of reference sample sex and country had no directional effect. In the performance testing, reference sample specificity did not consistently affect performance of the model. In the age estimation testing, slopes and  $R^2$  values of true age versus maximum likelihood estimates showed minimal differences between training/test sample combinations.  $R^2$  values ranged from 0.84 to 0.91, and slopes were between 0.94 and 1.03, indicating strong correlations with minimal bias. Absolute distance of the success rate from 0.95 was moderately negatively correlated with the size of the training sample ( $\rho = -0.5761$ ,  $p = 0.0499$ ). The width of the estimated age interval (scaled by true age) was not significantly correlated with the size of the training sample. Larger reference samples therefore produced equally precise age estimates that were more likely to match the expected error rate.

In conclusion, reference sample size is more important than homogeneity for producing age estimation models with low standard error. Furthermore, using a reference sample that is specific by sex and nationality does not produce consistently more accurate, more precise, or less biased age estimates than pooled reference samples.

### Population Specificity, Skeletal Development, Method Evaluation



## A78 Skeletal Age Estimation in the Living: Conventional Radiography (CR) Versus Magnetic Resonance Imaging (MRI) and Staging Technique Versus Atlas Method

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**Learning Overview:** After attending this presentation, attendees will understand why MRI is of added value to study the clavicles but not the wrist for age estimation in living adolescents and young adults. Additionally, attendees will understand why a wrist staging technique is more useful than an Atlas method for age estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing well-founded argumentation on the preferred approach for age estimation in the living based on developmental changes of the left wrist and both clavicles in terms of a comparison between CR versus MRI and staging technique versus the Atlas method.

**Background:** At present, forensic age estimation procedures in living adolescents and young adults rely on CR or Computed Tomography (CT) to visualize developing anatomical structures.<sup>(1)</sup> Hand/wrist and clavicle development both contribute to the age estimate during the considered age range. Nevertheless, in several countries, only CR is considered for skeletal age estimation, while international recommendations advocate CT to visualize the clavicles.<sup>1,2</sup> Moreover, MRI has been proposed as an alternative to CT to avoid exposure to ionizing radiation. Thus, a well-founded argumentation is needed to convince policy makers of the added value of MRI.

Skeletal development is assessed by allocating stages or Atlas standards, based on criteria of particular staging techniques or Atlas methods, respectively. Although Atlas methods seem more comprehensive, it is still unclear whether they should be preferred over staging techniques.

**Purpose:** To compare CR and MRI of the left wrist and both clavicles for forensic age estimation in living adolescents and young adults. The following hypotheses were made: (1) MRI outperforms CR, and (2) an Atlas method outperforms a staging technique to assess hand/wrist development.

**Materials and Methods:** CR and 3 Tesla (3T) MRI were prospectively conducted in 108 healthy Caucasian volunteers (52 males, 56 females) with ages ranging from 16 to 21 years. Five observers allocated stages and standards to (part of) the images independently. Staging techniques were applied to the left radius and ulna and to both clavicles.<sup>3,4</sup> Furthermore, Atlas methods were applied to the left hand/wrist.<sup>5,6</sup> For clavicle CR, one posteroanterior and two oblique radiographs were assessed simultaneously. Inter- and intra-observer agreements were quantified, and descriptive statistics were reported.

**Results:** Inter- and intra-observer agreements for wrist CR and MRI were similar. By contrast, the CR Atlas method was less reproducible than the staging technique. Inter- and intra-observer agreements for clavicle CR were lower than those for MRI.

Regarding the wrist, within-stage age distributions were similar on CR and MRI, as were those for the staging techniques and Atlas methods. Regarding the clavicles, the possibility to apply (profound) substages to MRI rendered a more gradual increase in the age distributions with increasing stages than on CR.

**Conclusion:** For multi-factorial age estimation based on the left wrist and both clavicles, CR suffices for the wrist, while MRI is necessary for the clavicles. Furthermore, a wrist staging technique is more useful than an Atlas method.

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### Age Estimation, Conventional Radiography, Magnetic Resonance Imaging

## A79 The Effects of Measurement Properties and Underlying Human Variation on FORDISC® Classification and Tolerance for Craniometric Error

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**Learning Overview:** After attending this presentation, attendees will better understand how modeling techniques can elucidate the impact of complex variable interactions on skeletal sex and ancestry assessment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new insight into patterns of misclassification in FORDISC® and new insight for generating appropriate tolerances for craniometric measurement error.

This research uses iterative manipulation to model the effects and interplay of craniometric measurement properties and underlying human variation on FORDISC® classification.<sup>1</sup>

Simulation techniques, such as the approach presented here, can help shed light on the sensitivity and validity of craniometric measurements used in discriminant function analyses, given that underlying variation and overlap in these traits within and among populations can strongly bias results when using small test samples. This study builds on previous work that employed similar methods with casework data by now modeling deviations from simulated “population-mean individuals” in order to parse out the effects of test sample or unknown individual “bias” from intrinsic measurement variables, such as magnitude and reproducibility, on misclassification.<sup>2</sup>

Twelve standard craniometric measurements (Biauricular Breadth [AUB], Basion Bregma Height [BBH], Cranial Base Length [BNL], Basion Prosthion Length [BPL], Maximum Cranial Length [GOL], Frontal Chord [FRC], Maxillo-Alveolar Breadth [MAB], Maxillo-Alveolar Length [MAL], Nasal Breadth [NLB], Nasal Height [NLH], Orbital Breadth [OBB], and Bizygomatic Breadth [ZYB]) were taken from the mean configuration of each of the 13 population groups in the Forensic Anthropology Data Bank (FDB, v1.24). These measurements were selected based on lowest Wilks lambda scores at Step 1 in a Forward Wilks lambda stepwise analysis using all FDB groups and all original DCP 1.0 measurements with the exception of mastoid height (due to issues with its measurement noted in DCP 2.0). For each group’s mean individual, an analysis was run using the above measurements and all FDB populations with no transformations. Then one measurement at a time was varied in 1mm increments ( $\pm 5$ mm) and reclassified. This process was repeated for all measurements, resulting in 121 runs per FDB group and 1,573 runs total. The classification and posterior probability results were recorded and compared.

Eighty-eight of the 1,560 (5.6%) deviation-based runs resulted in “misclassifications,” where error in one measurement changed the classification from that of the original mean individual’s group. Misclassification was roughly as likely to result from positive deviations as from negative deviations. Deviations  $\pm 1$ mm resulted in no misclassifications, while  $\pm 5$  mm affected misclassifications for all measurements except GOL and MAB—iterating these two measurements caused no misclassifications. GOL is the largest measurement included here, (grand mean = 178mm, range of means = 171–188mm), likely influencing its robusticity to perturbations; however, MAB is a “medium-sized” measurement with a smaller range of variation. The variables generating the greatest numbers of misclassifications were OBB and ZYB ( $N=18$  and  $15$ , respectively). Deviations of  $\pm 2$ mm resulted in misclassifications for NLB, NLH, and OBB, which constitute the three smallest measurements in every group and have the smallest ranges. Percentage deviations were also evaluated to standardize comparisons across craniometric measurements.

Parsing the results based on FDB group, WF had 0 misclassifications; WM, BM, AM, and BF groups also had low incidences ( $<2\%$ ). The robusticity against misclassification for WF likely stems from this population’s peripheral location in morphospace. Groups that misclassified most frequently were CHM and JM (20% misclassification rate each), followed by HM (10%). These misclassifications were often reciprocal among the three groups (e.g., +4mm deviation on AUB misclassified JM and CHM as HM, and -4mm deviation changed HM to a JM classification). These three groups started with the lowest posterior probabilities for their mean individuals, indicating large amounts of overlap in morphospace. Most misclassifications involved erroneous geographic/racial classification, with fewer instances of sex misclassification. However, all misclassifications of mean individuals from the AF or AM groups involved a switch of sex, and of the eight total instances of misclassified sex, seven involved the AF group.

The results of this study were compared to the results of an earlier study using casework individuals. The contrast in results highlights that the influence of measurement deviation on DFA depends on measurement magnitude, the range of measurement variation expressed by the reference populations, and where an unknown individual lies in morphospace. Together, these two studies suggest that blanket standards for tolerable measurement error are overly simplistic and the need to consider intrinsic measurement properties alongside patterns of human phenotypic variation when establishing guidance for measurement error tolerances.

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### Metric Analysis, Modeling, Error Tolerance

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## A80 Tracing Latinx Population History Through Odontometrics

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**Learning Overview:** After attending this presentation, attendees will better understand the utility of odontometric data to explore the population history of a modern Mexican Latinx sample.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that odontometric data can augment the information gleaned from genetic, historical, and other skeletal data as it pertains to samples with complex population histories such as those of Latinx groups.

Latinx populations have been shaped through more than 500 years of genetic drift and gene flow events taking place since European colonization of the Americas. The genetic variation among the parental Native American, European American, and African American populations has led to phenotypic variation within modern Latinx populations that is minimally understood within a forensic anthropological context. 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; regional genetic trends within the United States and Mexico are also known for Latinx populations.<sup>1-4</sup> Cranial studies have shown that the genetic variation within these populations is reflected phenotypically.<sup>5-8</sup> The few dental morphological studies on Latinx variation have also demonstrated differences among these samples and those of their parental populations.<sup>9-11</sup> Odontometric studies examining Latinx variation, however, are scarce despite the moderate to high genetic control of tooth size.<sup>12,13</sup> Given the rate at which people of Latinx descent are increasing within the United States, it is imperative that all available forensic anthropological methodologies be employed to understand the variation present within these populations.<sup>14</sup> This preliminary study examines tooth size from pre- and post-contact, as well as forensically significant populations to explore how microevolutionary forces have affected Latinx dentition. The goals of this research are: (1) to examine the variation within and contributions to modern populations among Native American populations, historic European/American, African/American, Asian, and Latinx populations; and (2) to test whether a modern Mexican Latinx sample can be distinguished odontometrically from other modern populations.

Samples within this study include North, Central, and South Native American skeletal remains from the Tsunehiko Hanihara Dataset, individuals from the San Gregorio Atapulco site housed at the Escuela Nacional de Antropología e Historia (ENAH) in Mexico City, a New Mexico Native American sample from the Phoebe A. Hearst Museum of Anthropology; historic European, Sub-Saharan African, Asian, Chinese, Ainu, Aleutian, and “Eskimo” skeletal remains from the Hanihara Dataset, individuals from the Santa Paula cemetery housed at ENAH; historic European American and African American data from the Robert J. Terry Collection; modern European American individuals from the Texas State University Donated Skeletal Collection, Japanese individuals from Chiba University, and Mexican Latinx individuals housed at the Universidad Nacional Autónoma de México (UNAM) in Mexico City. Mesiodistal and buccolingual crown and cervical measurements were taken as the maximum dimensions of the tooth for each of those planes following the definitions of Moorrees and Reed and Hillson and colleagues.<sup>15,16</sup> Following analyses for normality and sexual dimorphism, data were separated by sex. Measurements and data were narrowed to minimize missing data before imputation through *k*-nearest neighbor. Principal components analyses were utilized to reduce the imputed data; components with eigenvalues of 0.7 or higher were retained to capture more variation within the data.<sup>17</sup> Multidimensional scaling was utilized to visualize Mahalanobis  $D^2$  distances. Linear discriminant function analyses were run using odontometrics that met normality requirements and significantly differed among the modern samples in one-way analysis of variance tests with Tukey Honest Significant Differences corrections.

Results demonstrated similarities among the modern Latinx, historic Latinx, Native American, Asian/Asian-derived, and European/American samples for both the females and males. Modern female samples correctly classified with rates in excess of 54%, and male modern samples correctly classified with rates in excess of 60%. These preliminary results indicate that genetic drift and gene flow have shaped the tooth size of the Latinx samples under study, as anticipated by other forms of data but have also made them unique from other modern samples considered here. Additional research will seek to provide classificatory formulae for utilization by forensic practitioners.

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## Latinx, Odontometrics, Population History

## A81 Trotter and Gleser's (1958) Equations Outperform Trotter and Gleser's (1952) Equations in Estimating Living Statures of White Males

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**Learning Overview:** After attending this presentation, attendees will understand that Trotter and Gleser's (1958) equations perform better than those of Trotter and Gleser (1952) in estimating living statures of White males.<sup>1,2</sup> Additionally, attendees will learn that the Bayes factor is a useful tool to compare the performance of stature estimation equations quantitatively.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reassessing the utility of Trotter and Gleser's (1958) White male equations, which have long been underestimated and rarely used. It is also expected that the Bayes factor, which is used for comparison of equations in this study, will be widely utilized for a similar purpose in the forensic science community.

Trotter and Gleser presented two sets of stature estimation equations for White and Black males in 1952 and 1958. The 1952 and 1958 equations were based on World War II and Korean War casualties, respectively. Between the two sets of White/Black male equations, Trotter (1970) preferred the 1952 ones since their associated standard errors were smaller than those of the 1958 ones.<sup>3</sup> Since then, Trotter and Gleser's (1958) White/Black male equations have rarely been used, with no systematic validation tests. This research aims to re-evaluate the performance of the 1958 White male equations in comparison with the 1952 equations using quantitative measures.

Living stature and left long bone length data of 55 White male casualties, who were identified by the Defense POW/MIA Accounting Agency (DPAA), were used. Stature estimates of the individuals were produced using the 1952 equations, 1958 equations, and FORDISC®. When the "Trotter M Stats" option is selected, FORDISC® provides stature estimates based on Trotter's original data used for the 1952 study. The performance of the three methods was evaluated by three quantitative measures—bias ( $\sum(\text{estimated} - \text{actual})/n$ ), inaccuracy ( $\sum|\text{estimated} - \text{actual}|/n$ ), and Bayes factors. Good performance of a method is indicated by small bias and inaccuracy and large value of the Bayes factor. The 1958 equations mark the lowest bias of the three methods, except when the "femur+tibia" was used (0.32cm, 0.02cm, and -1.33cm for the 1958 equation, 1952 equation, and FORDISC®, respectively). As to the inaccuracy, the 1958 equations mark the lowest values in seven out of ten comparisons. Yet, in the comparisons where the 1958 equations did not mark the lowest ("tibia," "femur+tibia," and "femur+fibula" equations), the difference in the inaccuracy among the methods was minimal (<0.2cm). The greatest Bayes factors were obtained from the 1958 equations in all comparisons.

The Bayes factors allow for an objective comparison of performance among different equations by quantifying the similarity/dissimilarity of the distributions between the actual statures and estimated statures.<sup>4</sup> The fact that the greatest Bayes factors were obtained from the 1958 equations indicates that the distributions of the estimates by the 1958 equations are closer to those of actual statures compared to the other methods. Among the 1958 equations, those using the "tibia," "femur+tibia," and "ulna" yielded the greatest Bayes factors (BF's=7.0, 6.6, and 6.2, respectively); and those using the "humerus+ulna," "humerus," and "radius" yielded the lowest Bayes factors (BF's=1.5, 2.5, and 3.0, respectively). In general, the Bayes factor of 3 through 10 is interpreted as a "positive" or "substantial" evidence that the estimated statures by the equation follow the distribution of the actual statures.

The results of this study clearly show that the 1958 equations outperform the 1952 equations or FORDISC® in estimating statures of the White males. Also, it is necessary that researchers choose the best-performing equation based on an objective and quantitative measure such as the Bayes factor.

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### Stature Estimation, Equation, Bayes Factor

## A82 Metric Identification Support Tool (MIST): A Statistically Based Instrument for Use in Medical Examiner/Coroner Offices to Support Personal Identification Results

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**Learning Overview:** The goal of this presentation is to provide attendees with a major update on the continuing development of MIST, a statistically based instrument designed for no-cost online access for Medical Examiner/Coroner (ME/C) offices to use in personal identification of unknown decedents. Attendees will be able to assess the potential of this method of analysis that quantifies circumstantial evidence to support identification outcomes for individual decedents 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 demonstrating the current version of MIST and detailing the process by which pertinent circumstantial evidence pulled from medicolegal case information can be used in practice to mathematically assess the weight of this information in a personal identification case.

ME/C in the United States, who are responsible for the identification of unknown decedents under their jurisdictions, are hampered by the currently inadequate arsenal of identification methods. Most unidentified bodies receive a timely identification through fingerprints, dental records, or medical records, or the ME/C experience a longer turnaround time but receive successful results in cases requiring DNA analysis. However, many decedents remain unidentified for decades when the above-listed methods cannot be used or are inconclusive. In answer to this deficit, 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>

MIST, introduced to an AAFS audience in 2020, is 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). In 2019, a database of qualitative information gathered from 5,000 de-identified medical examiner cases was submitted to the project for the development, testing, and validation of MIST. The results from the early MIST development process and an automated preview of the instrument were presented at the 2020 AAFS Meeting. Once fully developed and operationalized, MIST will be a valuable tool in personal identification that applies statistical weight to the available qualitative evidence.

Progress on MIST has continued over the past year. Methods have been modified to use fuzzy numbers and logic. Fuzzy mathematics was developed to help quantify everyday imprecision and measurement uncertainty; the seminal paper was by Zadeh.<sup>2</sup> For example, “decedent stature is 70” from a postmortem exam might be replaced by an estimation of height using a bell curve to describe uncertainty in the decedent’s height. An eyewitness description of a missing person as “heavy-set” could be restated as “between 200 and 275 pounds.” Imprecise descriptors like “red-headed” or “looked Hispanic” might also be quantified. Fuzzy logic is a mature discipline, reducing the time needed for further MIST development. In addition to the incorporation of fuzzy logic into MIST when comparing the features of a missing person and the features of a decedent, a measure of likelihood has been added, based on distance between the location of the body and the residence of the missing person. Research efforts now focus on: (1) how best to quantify imprecise verbal descriptions, and (2) using simulation to determine the best ways to combine multiple descriptive features into an overall quantification of matching that allows useful ranking of candidate missing persons to a decedent (or vice versa). Examples include geometric mean, minimax (rank by weakest matching feature), and maximin (rank by strongest matching feature).

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### Identification, Forensic Anthropology, Forensic Pathology

## A83 An Introduction to a Skeletal Series for Forensic Anthropology Research and Training

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**Learning Overview:** Attendees will gain an appreciation of the Skeletal Series as a newly available documented skeletal collection for research and training. The history of the series as well as the demographic profile, pathologies and trauma, and other institutional resources are outlined. After attending this presentation, attendees will be familiar with the Skeletal Series as a valuable resource for support of skeletal research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the Skeletal Series, a valuable new addition to a limited group of modern documented skeletal collections. The Skeletal Series provides a new opportunity for forensic practitioners to train, research, develop methods, and validate existing methods. The Skeletal Series will continue to grow and diversify in terms of demography, pathologies, and trauma representation, making it an invaluable resource to the discipline.

This presentation introduces the Western Michigan University Homer Stryker MD School of Medicine (WMed) Skeletal Series, a new documented skeletal collection. The skeletal series consists of individuals that donated their bodies to support research and education, and forensic samples retained during postmortem examination at the Office of the Medical Examiner and Forensic Services. The Body Donation Program started in 2014 primarily to support medical education at WMed. From 2014 through 2019, a total of 222 people have donated their bodies to the program. There are several options for donation, and Forever Skeletal Donors consent to have their skeletal remains curated at WMed for future research and education. At the end of September 2020, a total of 102 donors have elected to become part of the skeletal series. Forty-three cadavers are still in use for anatomical instruction, and 59 are cleaned and maintained in the skeletal series. The age range represented in the skeletal series is 0–97 years, with most donors between 40–89 years of age at death. There are currently 17 (28.8%) females and 42 (71.2%) males in the series. Information on both ancestry and race are also requested from donors. Self-reported ancestry is broken down by continent: 2 (3.4%) African; 1 (1.7%) African and European; 2 (3.4%) American Indian; 2 (3.4%) American Indian and European; 1 (1.7%) Asian Indian; 44 (74.6%) European; 1 (1.7%) Mexican; and 6 (10.2%) Unknown. Self-reported race is comprised of 2 (3.4%) American Indian; 1 (1.7%) Asian Indian; 2 (3.4%) Black, 1 (1.7%) Hispanic; 50 (84.7%) White; 1 (1.7%) White and Black; and 2 (3.4%) Unknown.

At present, the program limits donations to individuals that have registered themselves in advance. There are 557 registered body donors, and 226 (40.6%) have selected the skeletal series as their final disposition. The registered donors are comprised of 125 (55.3%) females, 100 (44.2%) males, and 1 (0.004%) person who declined to report sex. The birth years for registered donors span 1924 to 1990, and the race and ancestry profiles are approximately the same as current donors.

Lifestyle and medical histories, including medical records, are available for most individuals in the skeletal series. These records often include antemortem radiographic and Computed Tomography (CT) images as well as postmortem radiography. Up to 58 metadata variables are also available for query in the donor records. Starting in 2021, information on an additional 30 variables will be collected. A wide variety of pathological conditions and traumatic injuries are represented in the skeletal series. These include osteoarthritis, erosive arthropathies, scoliosis, neoplastic diseases, acute and healed blunt trauma, high-velocity projectile trauma, and surgical interventions, including joint replacements and surgical implants.

In addition to skeletal material from body donors, the WMed Skeletal Series also includes retained skeletal samples from over 50 forensic cases. Infants to older adults are represented in the forensic subset of the skeletal series. These specimens comprise examples of blunt force, sharp force, and high-velocity projectile trauma from the perimortem interval and in various stages of healing. The majority of traumatic injuries are well documented. They present an excellent opportunity for training and for comparison to, and validation of, trauma studies.

The WMed Skeletal Series includes the skeletal remains of body donors and prosecuted forensic samples. The diverse and well-documented nature of the skeletal series makes it optimal for supporting research and training. This presentation offers an overview of the resources available at WMed, as well as the process for soliciting access to the skeletal series.

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### Documented Skeletal Series, Trauma, Methods

## A84 Bodies Without Names, Names Without Bodies: Searching for Lost Lives in Lisbon, Portugal

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**Learning Overview:** After attending this presentation, attendees will be aware of how identification is being done in Europe, namely in Portugal, and the reality of unclaimed corpses and unidentified bodies in Lisbon, which is becoming a major international metropolis. Attendees will also be aware of how important the complicity of forensic anthropology and pathology is.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by imparting how identification is performed and how the existence of databases of missing persons and unclaimed bodies is a major value for the current global world. Forensic anthropology is playing an increasing role in identification.

**Introduction:** In Portugal, the number of people who cannot be identified at the time of death, John and Jane Does, has not been thoroughly studied. The same applies to unclaimed corpses, that is, the bodies that are kept in the mortuary refrigerators for more than a month. Although there is an empiric suspicion that these numbers are increasing, we cannot be certain unless this is demonstrated. Furthermore, this is a crucial issue with social implications. Thus, this reality needs to be monitored, among others, to discuss the need to create a national resource center for missing, unidentified, and unclaimed person cases. The measures that should be taken to fight this social problem require that the reality is known. Moreover, this will be a better tool to identify foreign citizens whose numbers are currently increasing.

**Objectives:** This study is the beginning of a larger project, which aims to estimate the annual number of unidentified persons and unclaimed bodies/remains. It seeks to recognize demographic and social characteristics associated with dying and remaining unidentified to determine if the rates vary over time and if causes of death can explain any of these numbers. This study also seeks to answer questions such as: How many bodies were found across Lisbon and never named? Furthermore, how are bodies identified? Anthropological cases are included.

**Material and Methods:** This study presents an observational, retrospective, and descriptive study of unidentified and unclaimed bodies/remains over a three-year (2015–2017) period in Lisbon, Portugal, at the South Branch of the National Institute of Legal Medicine and Forensic Sciences (DS-INMLCF). The data were obtained from the DS-INMLCF database and archive. Microsoft® Office Excel® was used for the statistical analysis.

The techniques used to perform this complex forensic challenge, which is the identification of bodies and remains, are provided.

**Results:** The number of cadavers or remains arriving at DS-INMLCF with no identity amounts to 113 (3.06%) of the 3,692 admitted cadavers. All were submitted to examination, but nine were excluded from the analysis since there was no identification purpose: six were not human and three had no forensic interest. Of the 104 analyzed cases, 85 (81.73%) reached positive identification, in a time period ranging from a few hours to 780 days, with forensic anthropology and genetics having a crucial role, as well as fingerprinting analysis. Identified cadavers' ages ranged from 16 to 90 years, with a median of 49 years, and about a quarter were foreigners. Regarding the causes of death, 54.81% were violent, most of them due to accidents.

The number of unclaimed corpses was 155 (4.21%) out of 3,679 (13 cases were excluded as the remains were unclaimable: two fetuses under 24 weeks of gestational age, two exhumations at cemeteries, and the nine cases previously referred). The number of such cases grew over the three years studied. Cadavers' ages ranged from 20 to 109 years, with a median of 67.5 years; 12.26% were foreigners and the majority (75.48%) were males. The unknown bodies accounted for 23 (14.84%). Concerning the causes of death, 50.97% were natural.

**Conclusions:** Unlike the number of unidentified corpses, that has remained constant over these three years, the number of unclaimed ones has been increasing. The high rate of unclaimed corpses (4.21%) may be explained by their advanced age and socioeconomic conditions and entails both social and financial problems.

Although this study covers a short time period, the percentage of unidentified corpses and the increasing number of unclaimed ones reinforces the need to create a national research center for missing persons and unidentified and unclaimed cadavers. This center could allow a proper communication channel for professionals involved and an easily accessible database for the general population that would facilitate the recognition and claim of these lost lives.

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### Identification, Unclaimed Bodies, Unidentified Bodies



## A85 Management of the Dead After Disasters from Aquatic Environments in the Asia and Pacific Region

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**Learning Overview:** The goals of this presentation are to: (1) identify the problem of drowning in the Asia and Pacific Region; (2) provide information on how the search, recovery, and identification of the dead from aquatic environments within 12 contexts from this region are carried out within the various jurisdictional frameworks; and (3) provide suggestions to help improve the search, recovery, and identification of the dead from the water.

**Impact on the Forensic Science Community:** People are drowning at alarming rates in the Asia and Pacific Region. The bodies of these individuals are not always recovered or identified, in addition to the multifold problems associated with ambiguous loss for their families. Twelve contexts within the region are analyzed. This presentation will impact the forensic science community by offering suggestions to help improve this situation.

Much has been written about the management of the dead, in particular with regard to humanitarian forensic action following disasters, as included in the interagency manual, *Management of Dead Bodies after Disasters*, but there has been less attention given to the search and recovery of the dead following disasters from the specific context of aquatic environments.<sup>1</sup> With approximately 180,000 drowning deaths per year in the Asia and Pacific Region according to the World Health Organization (WHO), there is ample need for the authorities and first responders to be skilled in the management of the dead from various water contexts.<sup>2</sup>

As a result of natural disasters such as cyclones, tsunamis, earthquakes, volcanic eruptions, and floods, as well as human-made/influenced disasters such as boats capsizing and dam collapse, for example, the deceased can and do end up in the water. Aquatic conditions frequently complicate the search and recovery of the dead. Furthermore, the chances of recovery may be limited, and decomposition rates may be either accelerated or decelerated, when compared to those on land, depending upon many factors. To help avoid ambiguous loss and protect the dignity of the dead, best possible practices should be applied that may involve specialized forensic procedures, including the use of cadaver dogs in boats and specialized body bags for water recoveries.

National jurisdictions dictate who is responsible for the search and recovery of the dead at sea and/or other aquatic environments and these can vary from marine police to coastguards and navies, among others. The situation in international waters, on the other hand, is less clear. This research focuses on 12 Asia-Pacific countries (Bangladesh, India, Indonesia, Malaysia, Pakistan, Papua New Guinea, the Philippines, Thailand, and Vietnam and three land-locked countries: Afghanistan, Laos, and Nepal). The aim is to address how various authorities are searching for and recovering the dead in the water. The areas addressed in this research include, for example, who is the authority responsible for searching and recovering the dead in aquatic environments? Are cadaver dogs an appropriate use in the search for the dead in still water, such as in Malaysia? Is autopsy mandated for drowning deaths, such as it is in Thailand? Are the Search and Rescue Zones that are designed to provide structure to help in the search and rescue of the living used as well to help in the search and recovery of the dead in these contexts?

While each country within this study has different policies or approaches to the management of the dead from aquatic environments, some minimum guidelines are suggested to optimize recoveries and thus the identification of the dead, so that ultimately the dead can be returned to their families. The results of this study suggest that the search and recovery of the deceased from aquatic environments should be managed by trained professionals. For the lay person that finds a dead body or body part in the water, it is recommended that a Global Positioning System (GPS) reading is taken of where the dead body/body part is located and this location relayed to the responsible authority. Additionally, the use of cadaver dogs may aid in water searches, and the use of specialized body bags designed for water recoveries may ease water recoveries. In conclusion, various contexts in the Asia and Pacific Region manage the search and recovery of the dead differently in the water. Although there is no “right” way to undertake this often-challenging, time-consuming, and dangerous work, it is nonetheless important to help families find closure with the knowledge that their loved ones have been successfully recovered.

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### Drowning Deaths, Dead Body Search and Recovery, Asia and Pacific

## A86 Stakeholder Forums: Applying a Social Science Strategy for Recognizing Policy Consensus Among Divergent Stakeholders on Transnational DNA Data Sharing in Missing Persons Identifications

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**Learning Overview:** After attending this presentation, attendees will have learned of a novel process for evaluating policy options among stakeholders using social science research strategies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees on the challenges, priorities, and potential solutions for DNA data sharing to enable identification of missing persons across national borders.

In United States border states, human remains appearing to be migrants take months or years to identify and are sometimes buried without collecting biometrics for identification. Governmental and non-governmental efforts to improve recovery efforts, identification processes, communication, and repatriation processes are ongoing. DNA-based identification strategies across borders relies upon the sharing of: (1) Family Reference Sample (FRS) DNA data, and (2) Unidentified Human Remains (UHR) DNA data. However, DNA data sharing is complicated by questions of sovereignty, privacy, consent, and national security. Both the Combined DNA Index System (CODIS) and non-CODIS missing persons databases are under-populated and disconnected, resulting in missed opportunities for identifications. Several efforts have emerged to improve missing migrant identifications, yet disagreements and miscommunications among stakeholders have slowed progress. Multiple efforts to convene stakeholders to resolve some of the DNA-based challenges have resulted in incremental improvements in processes and increased awareness; however, many challenges persist. This study sought to develop a research strategy focused on the use of DNA data for identifications to systematically assess: (1) the challenges stakeholders face; (2) any varying stakeholder priorities; (3) potential agreement on solutions; and (4) gaps in data necessary to devise policy. Two Stakeholder Forums were held—each modeled as a cross between a focus group and a community forum—to capture quantitative and qualitative perspective data from stakeholders. The composition of the two Forums was designed to maximize consensus and trust and to minimize conflict within each.

The two in-person Stakeholder Forums were held with 26 representative stakeholders selected from qualifying professionals from 15 groups: local law enforcement, federal law enforcement, intergovernmental law enforcement, Justices of the Peace, medical examiners and coroners, consulates, intergovernmental organizations, humanitarian organizations, migrant family advocates, anthropological investigators, DNA forensic laboratories, federal database stewards, DNA technology laboratories, governmental officials, and human rights attorneys. A total of one to four participant representatives from each of 13 of the 15 groups (excepting governmental officials and human rights attorneys) were successfully enrolled. The semi-structured Forums were preceded by an information session where one representative from each stakeholder group presented challenges and perspectives from their vantage. Participants completed a demographic and pre-Forum questionnaire prior to each two-hour Forum, which were both closed to the public and recorded under an Institutional Review Board (IRB) protocol. Attendees used pseudonyms during the Forum. Audience response devices collected data during each Forum to supplement the pre-Forum questionnaire. One written qualitative question was asked during each Forum. Conversations were transcribed and coded for themes, subthemes, challenges, actionable policy suggestions, and positive and negative statements. Cross-referencing enabled the illumination of commonalities between the two Forums. In total, 79 challenges and 36 solutions were identified and sorted comprising eight categories: UHR recovery/processing; education on processes; infrastructure and policies; FRS collection/processing; DNA data sharing; long turnaround times; communication; and funding. While 21 of these challenges were raised in both Forums (e.g., long turnaround times to obtain matches), only one solution arose in both Forums (i.e., hire more laboratory technicians). The pre-Forum and in-Forum questionnaires reflected some consensus including: (1) a majority of participants agreeing that UHR should be uploaded to CODIS and could also go to a non-CODIS laboratory; (2) support for training of non-law enforcement personnel as FRS collectors; and (3) general support for rapid DNA in some aspect of identifications.

At times, mission differences (e.g., national security vs. humanitarian) result in disagreements in policy matters and priorities. Often, personalities and misunderstandings fuel the discourse, preventing progress in tackling the challenges. In recent years, observing the policy challenges in improving DNA-based identifications for transnational missing persons, strong emotional agreement has been observed among all stakeholders that the challenges ought to be addressed, and yet there has been no meaningful action to correct the policy gaps. It is hoped that systematic analysis of empirical data from a wide variety of stakeholders will aid in the construction of a long-term policy scaffold to manage the priorities in addressing the many obstacles.

### DNA Identification, Policy, Social Science

## A87 Rib Fracture Analysis of Infant Cardiopulmonary Resuscitation (CPR) Methods Using Porcine Surrogates

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**Learning Overview:** The goal of this presentation is to create a more comprehensive understanding of how CPR-induced rib fractures are presented in infants and how CPR-related infant rib fractures compare to those sustained in conjunction with abusive trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive understanding of how CPR-induced rib fractures are presented in infants using two different techniques. This research benefits those in the fields of forensic anthropology, medicolegal practice, pathology, pediatric medicine, and anyone who attempts to differentiate the causation of infant rib fractures as therapeutic or abusive.

The purpose of this research is to recognize if infant rib fractures are distinguishable between those caused by CPR or violence. In 2005, the American Heart Association suggested that when performing CPR on an infant, instead of using one hand to perform compressions, two hands should be used to create a front-to-back compression.<sup>1,2</sup> Prior to this switch, infant rib fractures were uncommonly associated with CPR, and therefore, associating rib fractures exclusively with abuse was clearer. Since using the two-hand approach, reports of CPR-related fractures have increased in autopsy documentation.<sup>3,4</sup> Because of the similarity in hand position between two-handed CPR administration and abusive scenarios, the cause of rib fractures cannot always be confidently discerned between CPR and violence.

One-handed and two-handed CPR methods were performed on deceased piglets to act as proxy for human infants. Afterward, autopsies of the piglets were performed to determine the location and type of fracture that occurred, or did not occur, on each rib. Furthermore, these results were compared to an infant trauma database from Harris County Institute of Forensic Science. The similarities and differences were noted between rib fractures sustained from CPR and those sustained in conjunction with abusive trauma.

This research demonstrated that both methods of infant CPR can produce rib fractures. Results from the piglet sample indicate that two-handed CPR causes statistically more rib fractures than one-handed CPR. Results from this study contradict current literature that uses descriptive terms like “highly unlikely” or “rare” to describe infant CPR-induced rib fractures.<sup>5-8</sup> The location of fractures identified on the piglet sample were observed exclusively on the anterior portion of the ribs, while fractures associated with abuse were observed most commonly on the posterior portion of the ribs. The pattern of rib fractures following CPR is distinct and will aid in differentiating from patterns more typically associated with child abuse.

This animal model research has direct application to a significant set of social and legal issues with conclusions that can be relied on by biological forensic anthropologists conducting medical-legal death investigations. Further benefit is to fields of law enforcement, pathology, pediatric medicine and anyone who attempts to differentiate the causation of infant rib fractures as therapeutic or abusive. Interpreting whether an injury is a result of child abuse or therapeutic intervention is a stressful and precarious situation. Cases may not present with obvious causes of injury, and therefore diagnostic evidence is crucial. The expectation of this research was to create a comprehensive understanding of how CPR-induced rib fractures are presented in infants.

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### Rib Fractures, Infant, CPR

## A88 Experimentally Induced Rib Fractures Using Euthanized Burmese Pythons (*Python Bivittatus* Kuhl): A Comparison of Compression Impacts to Inform Pediatric Rib Fractures in Humans

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of rib fracture location as a consequence of experimentally induced compressive forces using euthanized Burmese python thorax sections to inform pediatric rib fracture analysis for infants 0 to 1 year of age.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence for interpreting rib fracture location consistent with anterior to posterior compression and posterior to anterior compression using Burmese pythons (*Python bivittatus* Kuhl) of size and thorax dimensions similar to human infants from birth to 1 year to inform cases of pediatric rib fracture analyses.

In Florida, forensic anthropologists assist medical examiners and child protection investigators by assessing fracture patterns in dead and living pediatric patients, respectively. Since the “... possibility of child abuse is often overlooked in clinical practice,” forensic anthropologists tend to be called more often to assist medical examiners with pediatric autopsies because both healed and acute injuries inform the medical examiner’s analysis of cause and manner of death.<sup>1</sup> Even with the inclusion of the forensic anthropologist at autopsy, distinguishing acute accidental from acute non-accidental injuries is difficult and may result in confusing findings.

Physical child abuse has been linked to extrinsic stressors such as poverty and un- or underemployment of parents and caregivers.<sup>2</sup> Importantly, both extrinsic variables have risen with the COVID-19 pandemic, thereby making research that investigates methods that help to identify pediatric fractures at autopsy timely and important. Until now, most research into the etiology of blunt skeletal fractures have involved experimental studies on non-human exemplars (*S. scrofa* or pig and *O. cuniculus* or rabbit) and meta-analyses using clinical and medical examiner cases.<sup>3-5</sup>

In 2017, Florida Gulf Coast University’s (FGCU) Human Identity and Trauma Analysis program partnered with the Conservancy of Southwest Florida as part of a larger project to remove invasive Burmese pythons from the Everglades. Using Burmese pythons as an exemplar for human infant cadavers (between birth and 1 year of age), an Instron® 5544a apparatus (1,500mm/min) was used to induce anterior ( $N = 30$ ) and posterior ( $N = 30$ ) compressions on the python samples ( $N = 60$ ). Fracture location was recorded following Love and colleagues to find and determine if the exemplar fracture location patterns correlated to those seen in infants with accidental and non-accidental injuries to the thorax from published research.<sup>6</sup> In addition to the four regions of classification by Love and colleagues (anterior, anterolateral, posterolateral, and posterior), a fifth region was included, for fractures located laterally where the anterolateral and posterolateral regions meet.

Results of the experiment revealed 28 of the 60 total samples (46.6%) presented with rib fractures. From the 30 anterior compression tests, 13 samples (43.3%) presented fractures (total = 34) located on the anterior ( $N = 12$ ), anterolateral ( $N = 13$ ), lateral ( $N = 3$ ), and posterolateral ( $N = 6$ ) aspects of the ribs. Posterior compression tests revealed that 15 of the 30 samples (50%) presented with fractures (total = 24) located on the anterior ( $N = 6$ ), anterolateral ( $N = 1$ ), lateral ( $N = 4$ ), posterolateral ( $N = 12$ ), and posterior ( $N = 1$ ) aspect of the ribs. A total of 1,438 ribs were observed, with only 54 ribs (0.04%) having fractures. These findings were consistent with previously published research that found that anterior to posterior manual compression (such as cardiopulmonary resuscitation) presented anterolateral or anterior fractures.<sup>7</sup> In addition, this study evidenced posterior and posterolateral rib fractures from posteroanterior compression, as expected.

This study fills a gap in the research by experimentally inducing rib fractures using non-human exemplars, *Python bivittatus* Kuhl (Burmese Pythons), that allow multiple samples to be used from one specimen. Specifically, the long bodies of these euthanized invasive species allow 8–10 rib cages comprising 24 ribs of the same size to be impacted. By ensuring the continuity of the rib sections, this study created a sample comparable to the sizes of infant thoraxes. In addition, while this research sheds light on the likelihood that distinct rib fracture patterns may enable analysts to indicate whether the impact was from the front or back of the infant, these unexpected results indicate that caution should be used when interpreting fracture patterns and even more so when extrapolating from non-human exemplars to human remains.

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### Pediatric, Rib Fracture, Compression

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## **A89 Differentiating the Skeletal Trauma Resulting From Pediatric Simple Short Falls Compared With Physical Abuse: A Retrospective International Multicenter Pilot Study**

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**Learning Overview:** After attending this presentation, attendees will have an improved understanding of the differences in skeletal fracture patterns and types that result from Simple Short Falls (SSFs) compared with physical abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by strengthening the evidence base for the interpretation of traumatic injuries resulting from blunt impact loads in complex fatal pediatric cases. This presentation will also highlight the value, and necessity, of multidisciplinary and multicenter approaches to trauma analysis in forensic anthropology and create awareness of the Registry of Pediatric Fatal Fractures (RPF).

Differentiating the skeletal trauma that results from accidental SSFs with physical abuse is complex, as both events result in a blunt impact load and thus are biomechanically alike. Due to the relative rarity of these events resulting in fatality, the evidence base for skeletal trauma resulting from these blunt impact loads has remained limited to anecdotal studies; with many of the current findings non-substantiated or unclear. As such, it remains difficult to differentiate the skeletal trauma resulting from fatal SSFs with fatal physical abuse blunt impact loads. The aim of this pilot study was to use a multicenter approach to investigate if there were differences in the patterns and types of fractures resulting from physical abuse and SSF blunt impact loads.

Cases of fatal pediatric (<10 years) blunt impact loads resulting from physical abuse and SSFs ( $\leq 1.5$ m) were retrospectively collected from the Victorian Institute of Forensic Medicine (Australia), Institut Médico-Légal de Paris (France), Great Ormond Street Hospital (England), and University of Pretoria (South Africa). Postmortem computed tomography scans and/or skeletal surveys were reviewed for each case to document the location and morphology of fractures. Intrinsic and extrinsic variables were recorded from forensic pathology and radiology reports, police reports and, where relevant, coronial findings. Fractures were analyzed using descriptive statistics.

Five SSFs and 18 physical abuse cases met the study inclusion criteria. Fractures occurred in 2 (40%) of the SSFs and 11 of the physical abuse blunt impact loads. The SSF cases exhibited fractures only to the skull, while physical abuse cases exhibited cranial and post-cranial fractures; 73% ( $n=8$ ) located only in the skull, 9% ( $n=1$ ) only in the post-cranial, and 18% ( $n=2$ ) located in both. In the cases of SSFs, only a single skull bone fractured as a result of the blunt impact, while in the physical abuse cases, fracturing involved more than one skull bone in 60% of the cases with skull fractures. Skull fracture types were simple linear in cases of SSFs, while comminuted, simple linear, multiple linear and diastatic fractures were found to result in the physical abuse cases.

Fatal blunt impact loads resulting from SSFs and physical abuse are rare events. Given the relative rarity of such cases, this pilot sample was small and, consequently, conclusions drawn from the data may only be considered anecdotal. At the simple descriptive level, fractures identified in this pilot study strengthen the current anecdotal evidence base that, while different types of fractures may occur to all aspects of the skeleton in cases of physical abuse blunt impact loads, typically only linear fractures of the skull result from SSF blunt impact loads. To move beyond an anecdotal evidence base, the data collected from this pilot ( $n = 23$  cases) form the foundation of the RPF. Augmentation of the RPF with additional multicenter contributions would allow the descriptive findings of this pilot to be validated.

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### **Pediatric Skeletal Trauma, Blunt Impact Load, Multicenter Trauma Registry**

## A90 Differences in Fracture Healing Between Unintentional and Abuse-Related Fractures in Young Children

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**Learning Overview:** After attending this presentation, attendees will recognize the potential effect of physical abuse on fracture healing in young children and its impact on assessment of fracture age in cases of suspected child abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the results of this study that has the potential to impact the application of fracture healing timelines when assessing fracture age in cases of suspected abuse.

Young children (0–3 years) are most vulnerable to death as a result of abuse and maltreatment.<sup>1</sup> Children under one year of age are at the highest risk for physical abuse and have the highest number of fatalities due to maltreatment.<sup>1</sup> Over half of child physical abuse cases involve skeletal fractures, and the presence of multiple fractures in varying stages of healing may be highly concerning for physical abuse.<sup>2,3</sup> An accurate time-since-injury estimation of healing fractures may provide an opportunity for identification and characterization of physical abuse, especially in young children unable to verbalize. Timelines of fracture healing are primarily derived from unintentional (i.e., accidental) fractures in otherwise healthy children and applied to children suffering physical abuse and other forms of maltreatment. The objective of this study was to examine the potential effect of abuse on fracture healing in a sample of young children and the predictive potential of fracture healing data.

This Institutional Review Board (IRB) -approved retrospective, radiographic study examined children under two years old evaluated for a humerus fracture between 2000–2016 at a pediatric level I trauma center. Humeral fractures in immobile infants without a known mechanism of injury are considered highly concerning for abuse but are also seen as a result of birth-related trauma and unintentional injury.<sup>4</sup> Exclusion criteria included: (1) casted radiographic exams, if the fracture was obscured on all views; (2) unknown date of injury; (3) individuals with known comorbidities or disorders affecting bone; and/or (4) fractures requiring instrumentation. Features of fracture healing (Subperiosteal New Bone Formation [SPNBF] and callus formation) were evaluated from initial and follow-up radiographic exams. Determination of abuse was made by the hospital's child protection team; however, not all children in the sample were necessarily evaluated. Kruskal-Wallis H tests were performed to determine if there were significant differences in fracture healing time between abuse-related and unintentional fractures. Building on previous research that demonstrated a difference in fracture healing time between abuse-related and unintentional fractures, mixed effects models were developed to investigate influences of fixed and random effects and predictive potential of these data.<sup>5</sup>

Fifty-six humeral fractures (abuse-related: 19, unintentional: 37) were analyzed from 63 patients (males: 31, females: 32). Twenty (36%) were infants less than one year and 36 (64%) were age 1–2 years. A total of 76 abuse-related and 100 unintentional radiographic exams were analyzed, with an average of three post-injury exams per patient (abuse-related: 4, unintentional: 2.7). Exams were performed a mean of 19.95 days (Standard Deviation [SD]=23.81) post-injury for abuse-related fractures and 19.35 days (SD=19.71) post-injury for unintentional fractures. Most abuse-related fractures occurred in infants and were complete and diaphyseal. Multiple fractures were observed in over half ( $n=11$ , 58%) of children with abuse-related fractures and three children (8%) with unintentional fractures. SPNBF and all levels of callus matrix occurred earlier in abuse-related fractures, but only intermediate callus approached statistical significance ( $p < 0.05$ ). Clinical/forensic relevance (as opposed to statistical significance) should be considered. While a difference of two days is unlikely to make a significant difference in fracture age estimation, a difference of 7–10 days may have implications for identification of abuse. Results from the mixed effects models demonstrate that abuse-related fractures heal slightly faster than unintentional fractures at a fixed rate of 3.4 days when indicators of healing are controlled. Equations generated from the final model predict time since injury and have the potential to discriminate abuse status in the middle and advanced stages of healing.

The population from which a fracture healing timeline is derived (healthy children) and to whom the method is applied (abused children) should be considered. In addition, equations to predict time since injury may assist in identification of physical abuse in children.

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### Fracture Healing, Child Abuse, Time Since Injury

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## A91 The Effects of Input Energy and Impact Surface on Cranial Fracture Patterns

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**Learning Overview:** After attending this presentation, attendees will have learned about cranial fracture patterns generated in human cadaver head impact experiments performed at two levels of input energy with three impact surfaces of different shapes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing documentation of experimentally generated human cranial fracture patterns. These data provide a baseline for understanding the influence of input energy and impact surface shape on cranial fracture production and evaluating fractures in forensic cases.

Several extrinsic variables are expected to influence cranial fracture patterns, including the magnitude and duration of force, impactor mass and velocity, and the contact surface between an implement and skull. While these relationships may be theorized using thought experiments, experimental data can help link these mechanical inputs with fracture outputs.<sup>1</sup>

This study examines the effects of two extrinsic variables: input kinetic energy and impact surface, on cranial fractures. Previously, experiments were performed on 12 Postmortem Human Subject (PMHS) heads using three implements with different contact surfaces.<sup>2</sup> The current study builds on this work to assess fracture patterns obtained at a comparatively higher input energy. This study aimed to: (1) document mechanical response and fracture data for these higher-energy experiments, and (2) compare results obtained in the current study to those from previous experiments to investigate the effects of input energy and impact surface on fracture behavior.

Mid-parietal impact experiments were performed on 12 unembalmed, upright PMHS heads. The impact methodology has been described in detail in a previous publication.<sup>2</sup> Input energy was increased relative to the original experiments primarily via the addition of impactor mass. The same three implements with different contact surfaces were used in the current study: a small, focal surface (1.1-inch diameter cylinder base); a broad, curved surface (length of a 2.5-inch diameter, 2.5-inch long cylinder); and a broad, flat surface (3-inch diameter cylinder base). Characteristics of the resulting fractures were assessed for each experiment. Maximum Defect Diameter (MDD) of non-linear defects was measured with sliding calipers.

The average input energy ( $E_i$ ) of the current experiments was  $180.07 \pm 23.02$  J, which represented a 67% increase in  $E_i$  from the previous experiments ( $106.11 \pm 8.80$  J). While the average energy absorbed by the head ( $E_a$ ) was significantly higher in current ( $86.31 \pm 25.31$  J) versus previous ( $55.21 \pm 10.35$  J) experiments ( $p=.0007$ ), there was no statistically significant difference in average peak force obtained in higher-energy ( $6980.8 \pm 2036.9$  N) versus lower-energy ( $6415.82 \pm 2155.92$  N) experiments ( $p=.52$ ).

The results indicated greater complexity and severity of fracture in higher-energy versus lower-energy impacts. Crania in the higher-energy group exhibited depressed and comminuted fractures more frequently than those in the lower-energy group. Only the higher-energy impacts produced fractures crossing the midline. However, overlaps in the results obtained between energy groups, particularly with broader impact surfaces, suggested that the degree of damage might be substantially influenced by individual properties of the cranium.

The results also suggested differences in the appearance and size of defects based on contact surface. The focal and curved surfaces more frequently produced localized depressed and circumferential fractures, whereas the broad, flat surface more frequently produced patterns involving only linear fractures. The flat surface (MDD= $63.15 \pm 7.21$ mm) produced larger defects than the focal surface (MDD= $35.02 \pm 10.25$ mm), while the curved surface produced defects intermediate in size (MDD= $47.21 \pm 19.45$ mm). However, all three implements produced defects larger and/or smaller than the impact surface diameter. Variation in contact area is one factor that could explain this result. The three surfaces tested have different expected contact areas, but individual factors such as cranial curvature may affect the actual area of contact between these surfaces and a head.

The size and appearance of defects obtained in this study supported the general conclusions that focal, penetrating depressed fractures can likely be associated with similarly focal surfaces, whereas larger circumferential and depressed fractures likely indicate broader surfaces. However, the relationship between impact surface and defect size is highly variable and therefore cannot be precisely reconstructed, especially in the case of broad defects.

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### Trauma Analysis, Cranial Fracture, Blunt Force Trauma

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## A92 The Issues and Complexities of Establishing Methodologies to Differentiate Between Vertical and Horizontal Impact Mechanisms in the Analysis of Skeletal Trauma

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**Learning Overview:** After attending this presentation, attendees will understand the difficulties of interpreting the mechanism of Blunt Force Trauma (BFT) skeletal trauma resulting from catastrophic loading events when minimal contextual information is available. There is a significant amount of literature documenting the complex relationships between impact (force, mechanism, etc.) and trauma; however, existing techniques/methods are limited when applied to medicolegal cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that new approaches are required to determine the relationships between impact and skeletal trauma outcomes in order to meet standards of evidentiary value in medicolegal cases.

**Background:** Understanding wound characteristics is fundamental in the investigation, interpretation, and assessment of BFT. Forensic practitioners frequently recover and analyze traumatic injuries in individuals presenting BFT without contextual information. The absence of contextual information can hinder interpretation and, for the forensic pathologist, can create uncertainty in ascertaining the most probable manner of death. Forensic anthropological interpretations are additionally complicated by variables such as the length of time since death, loss of soft tissue, and the changes that may mask or mimic trauma resulting from, for example, the removal of remains from the death scene and other postmortem taphonomic changes. Identifying the impact mechanism (high-impact falls, pedestrian Motor Vehicle Accidents [MVAs], accidental/intentional impacts, etc.) is important to accurately determine the manner in which the death occurred and can be a critical component of evidence given in courts.<sup>1</sup>

The mechanism of BFT is difficult to interpret on the basis of skeletal fractures alone.<sup>2</sup> Considerable variation exists within each type of injury dependent on the impact force, direction, and individual biological characteristics. The extent of injuries from both lateral and vertical deceleration events are influenced by several of the same factors mentioned above.<sup>3</sup> In medicolegal settings where there may be limited contextual information, forensic pathologists determine the most probable scenario based on the condition of the physical remains of the individual, where BFT impacts are distinguishable by soft tissue injury and underlying skeletal damage.<sup>4</sup> In forensic medicine, there are limited studies and replicable methodologies that facilitate objective assessment of traumatic injuries, either in terms of impact force or impact mechanism.<sup>5</sup> This fact raises concern regarding the evidentiary value of the current state of trauma interpretation. It also leads to significant doubt regarding whether quantification of skeletal trauma from impact mechanism is at all possible considering the complex non-linear behavior skeletal trauma can exhibit. Because of the difficulties in relating impact mechanism to injury (especially when attempting to determine accidental or intentional injury causation) when bone is severely traumatized, it leaves a void in medicolegal reasoning.<sup>6</sup>

**Goal:** Given the complex processes governing the nature of BFT skeletal injuries, determining whether differences between impact mechanisms and other factors (i.e., impact forces) can be quantified requires investigation.

**Aim:** This study aims to determine the feasibility of quantifying differences between impact mechanisms by using a formula created from transformed variables recorded from specific trauma cases involving BFT trauma to the femur.

**Methodology:** Displacement, comminution, and femoral midshaft area data were recorded from full body postmortem computed tomography scans of 103 individuals (males, mean age 42.5, and females, mean age 48.9) where cause of death was the result of rapid deceleration impact events, both horizontal (pedestrian MVAs,  $n=59$ ) and vertical ( $> 3$ -meter falls,  $n=46$ ). These measurements were standardized and transformed into a continuous variable. Independent  $t$ -tests, binary logistic regression, and K Nearest-Neighbors (KNN) were used to analyze the data.

**Results:** Mean group differences between falls (9.62) and pedestrian MVAs (9.53), were not statistically significant, while KNN models showed a 59.5% to 70.9% probability of predicting impact mechanism.

**Conclusions:** The results indicate that similarities in variance between types of trauma outcomes and impact mechanisms demonstrate low equivalency (samples have limited differences). Further, relying on using single elements to explain complex skeletal trauma outcomes is limited. The research undertaken in this project has shown the inherent complexities of analyzing skeletal trauma post-hoc, and that even with quantitative methods, statistically differentiating between impact classes remains elusive.

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### Fracture Analysis, Impact Mechanism, Forensic Anthropology



## A93 An Assessment of the Ability of Forensic Anthropologists to Detect Skeletal Trauma Using Radiological Methods

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the role of radiological methods in modern forensic anthropology, as well as the ability of forensic anthropologists to accurately detect skeletal trauma using imaging modalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and anthropology communities by highlighting the ability of forensic anthropologists, in terms of fracture-detection sensitivity and specificity, to identify blunt force trauma using various imaging modalities.

Forensic anthropological analyses are increasingly being conducted using radiological methods. These anthropological examinations often include the assessment of skeletal trauma, which is common in cases of physical abuse and violent crimes. However, while forensic anthropologists may be required to detect and comment on fractures using radiological material, they generally have very limited, if any, radiological training or experience. The aim of this study was therefore to assess the ability of forensic anthropologists to detect skeletal trauma using radiological means.

The skull, trunk, and limbs of five piglets were subjected to blunt force trauma using a mallet and were then scanned using Computed Tomography (CT), X-ray, and Lodox<sup>®</sup>. Each scan was read by a Senior Radiology Resident (SRR), a Novice Forensic Anthropologist (NFA) with brief training in the basics of radiological image interpretation, and more Experienced Forensic Anthropologists (EFA) with limited-to-no radiological training. Each observer recorded the number of fractures detected using each imaging modality, and the level of agreement between each of the observers was calculated using Cohen's kappa. In order to calculate the fracture detection sensitivity and specificity for each observer, the number of fractures identified by each observer using each radiological method was compared to the number of fractures detected on the dry bone, which was recorded following skeletonization of the piglets.

The level of agreement between all observers was considered fair to almost perfect, with kappa values ranging between 0.37 and 1.00. However, while sensitivities were similar between the SRR (73.6% for CTs, 52.1% for X-rays, and 46.5% for Lodox<sup>®</sup>) and the NFA (77.8% for CTs, 55.3% for X-rays, and 48.2% for Lodox<sup>®</sup>), an average of 22.7% less fractures were detected by the EFA. All observers detected more fractures using CT scans than either X-rays or Lodox<sup>®</sup>, which suggests that, irrespective of the level of observer experience, CT scans are easier to interpret than plain radiographs. All observers had very high specificities, which ranged between 95.1% and 99.2%, and were comparable between each radiological method. This suggests that level of training or experience does not influence the rate of false-positive fracture identifications using any imaging modality.

The results of this study indicate that training in the basics of interpreting radiological images improves the ability of the observer to detect skeletal trauma using virtual means. Without this training, the ability of forensic anthropologists to virtually detect fractures during forensic anthropological analyses may not be sufficient. It is therefore suggested that all forensic anthropologists should receive radiological training prior to conducting forensic anthropological examinations that include skeletal trauma assessments using radiographic material.

### Forensic Imaging, Forensic Anthropology, Virtual Trauma Assessment

## A94 Bone Diagenesis in the Marine Environment: Trace Element Distribution in Mammalian Bones Recovered From Shipwrecks

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**Learning Overview:** The goal of this presentation is to describe the principles of bone diagenesis and their potential application to forensic science, the distinction between biogenic and diagenetic chemical signals in bones, the correlation between macroscopic and chemical characteristics of bone diagenesis, and an example of trace element analysis in a set of archaeological bones submerged for a known length of time in a marine environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing how the establishment of a correlation between macroscopic alterations and trace element concentrations and distributions in bones recovered from a marine environment will prove essential for members of the forensic science community involved in taphonomic assessments.

Bone diagenesis is the global effect of the physical, biological, and chemical transformations that bones undergo between death and discovery in the archaeological or geological record. Diagenetic transformations, macroscopic and microscopic, are influenced by the physics, chemistry, and biology of the depositional environment. In living organisms, chemical processes are affected by diet, mobility, and pathologies. While studies of the diagenetic modifications and chemical composition of buried bones are extensively featured in the scientific literature, geochemical signatures characteristic of underwater bone diagenesis have not been studied in detail. This study investigated whether a geochemical fingerprint of the interaction between 11 archaeological mammalian bones and seawater and/or marine sediment could be discerned.

The analyzed mammalian bones belong to a museum collection of underwater archaeological materials excavated from four submerged shipwreck sites off the Western Australia coast: Batavia (1629), Vergulde Draeck (1656), Zeewijk (1727) and Rapid (1811). The underwater excavations were conducted between 1968 and 1980, and bones from the four wrecks had been submerged in seawater and/or sediment for 347, 316, 241, and 169 years, respectively. With one exception, all of archaeological bones were fragmented, some were also heavily stained, and in two samples, the damage to the protective cortical layer was particularly extensive. Bone trace element chemistry was compared to that of a modern sheep bone (*Ovis aries*). Laser ablation-inductively coupled plasma/mass spectrometry was undertaken across bones mounted in epoxy rounds. Cross-sectional spot transverses followed a path from the cortical layer (exterior) through the trabecular bone in the interior.

In the modern sheep bone, several trace elements showed bulk concentrations close to, or at, the limit of detection (Chromium [Cr], Cobalt [Co], Nickel [Ni], Copper [Cu], Yttrium [Y], Rare-Earth Element [REE], Thorium [Th], and Uranium [U]). In contrast, in the submerged bones, Lithium (Li), Cr, Cu, and U were elevated relative to the modern sheep bone, whereas Rubidium (Rb) and Barium (Ba) were depleted. Normalized trace element patterns in modern bone were flat, whereas in the archaeological samples, the normalized trace element pattern in the only whole sample (from Batavia) was different from that of the damaged bones from the other wrecks. Most elements with altered bulk concentrations in the archaeological bones are non-essential to biological life (Cu being the exception), supported by their low concentration in the modern sheep bone. However, Ba is usually enriched in bone by reason of known para-physiological metabolic processes. Since Li, Cr, Cu, U, Rb, and Ba are present in seawater in very low concentrations (<1ppm), it is reasonable to assume that in the archaeological bones, the relevant increase in bulk concentrations of Li, Cr, Cu, and U is entirely diagenetic in origin, perhaps due to protracted chemical exchange with sediment. The depletion of bulk concentrations of Rb and Ba is also diagenetic in origin and can be explained by protracted exposure to seawater and sediment. Furthermore, since the structure of cortical bone is denser than that of trabecular bone, cortical bone is less susceptible to alteration. This is reflected in the flat normalized element distribution profiles in bones where the cortical layer is missing or heavily damaged. As a consequence, the bulk chemical composition resulting from diagenetic chemical exchange in bone appears to be more uniformly distributed if the cortical layer is heavily damaged or missing, as reflected by the flat normalized elemental distribution profiles. In the only undamaged sample, the profiles of Li, Ba, Magnesium (Mg), Strontium (Sr), and Rb showed a gradual decrease in concentration from the outer surface toward the interior of the cortical bone. The overall conclusion is that macroscopic diagenetic alterations influence elemental concentrations and patterns of elemental distribution in bones, and their analysis allows the reconstruction of different taphonomic pathways.

### Bone, Diagenesis, Marine

## A95 Widespread and Common Blow Fly Species in the Northeastern United States: How Useful Are They in the Forensic Inference About the Movement of a Corpse

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**Learning Overview:** After attending this presentation, attendees will be familiar with past surveys conducted to examine seasonal and habitat preference of blow flies and the results of the current blow fly survey conducted in the northeastern United States. Attendees will also be exposed to the method used to obtain this data and the feasibility of forensic inference from blow fly distribution data and corpse relocation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising questions regarding the limits of distribution studies and ways to improve forensic inference in this area.

Forensic entomology is primarily used to aid in the estimation of time since death but is also used to assess if a corpse has been moved from a primary crime scene. For a relocation argument to be valid, individual blow fly species must show specific habitat and or seasonal preference. Past surveys have shown that species composition can vary across trap sites, and this has resulted in the categorization of individual species as a warm or cold weather species and as urban or rural. Past surveys have also shown, however, that many blow fly species are not clearly associated with a particular ecoregion, with habitat preference varying by region and very few species being found exclusively in a habitat category, such as urban or rural, or exclusively in a season. Native and non-native species differ in their flexibility to survive in a specific habitat or a cold winter, and most forensically important blow flies in the United States are considered non-native and widespread. Non-native species are more likely to behave as generalists, are opportunistic in terms of feeding and habitat, and are often highly synanthropic.

To determine if past categorizations of blow fly species can be applied to the local region, a survey of blow fly species was conducted in Connecticut. Connecticut is a northeastern state of the United States with little-to-no forensically relevant entomological survey data. Five traps were set over a two-year period to determine which blow fly species dominate the region and whether individual species show habitat or seasonal preference. Trap locations were selected near Hartford, a city with a high murder rate, and within secluded areas ideal for the disposal of a corpse. Geographic Information Systems (GIS) mapping was used to categorize a 1km radius around each site in terms of land use and degree of urbanization. Trap sites were shown to be heavily fragmented in terms of land use, and sites differed significantly in terms of the percent of developed land and degree of urbanization. Despite trap locations differing significantly in terms of the degree of urbanization and percent of developed land, an Analysis of Variance (ANOVA) ( $P < 0.05$ ) showed that the species of blow flies trapped at each site did not differ. No relationship was also found between the number of flies caught, location, and season.

A significant correlation was only found between the recorded temperature and the number of flies caught. Trapped specimens represented three genera and eight species, with 96% of trapped specimens represented by three species: *Phormia regina* (67%), *Lucilia coeruleviridis* (22%), and *Lucilia sericata* (7%). *Phormia regina* is the dominant carrion fly for most of the United States and is found throughout most of the northern continents of the world. It is considered a cold weather species with hemisynanthropic and exophilous synanthropy, and while primarily rural, is also found in urban areas and during hot summers. *Lucilia coeruleviridis* is likewise common in the United States and has a Nearctic distribution. The species lacks the recognition of other dominant species but is considered a predominant species in the spring and fall. *Lucilia sericata* is one of the most common and widespread species of *Lucilia* in the continental United States, is thought to be endemic to the Palaearctic and Nearctic regions, is cosmopolitan, and has been for some time. *Lucilia sericata* shows a preference for urban habitats, less tolerance for cold weather, and demonstrates eusynanthropic and endophilic synanthropy, however, this changes according to region and more specifically latitude.

Results from this current survey suggest that individual blow fly species in this region are flexible in terms of habitat and seasonal preference, with no clear preference for a particular ecoregion or season. Results also suggest that the degree of urbanization has no influence on habitat selection. Temperature was the only factor contributing to abundance, which is not surprising given that blow flies are ectotherms and temperature is the largest physiological constraint limiting their geographical distribution. This survey provides forensically relevant trap data that will be of use for future forensic entomology cases in this region. The results of this survey do not, however, categorize individual species in terms of habitat and seasonal preference. Blow flies have demonstrated their success and ability to adapt to changing environments, and their ubiquitous nature makes them an excellent resource for helping solve crimes. However, their flexibility and lack of a strict habitat and seasonal preference presents a challenge when trying to use their presence on a corpse to demonstrate the previous location of that corpse. Such results should also prompt investigators to be judicious when using past categorizations of blow fly species to argue movement of a corpse.

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### Blow Fly, Forensic Entomology, Corpse Relocation

## A96 Using Metabolomics to Gain a Deeper Understanding of Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will understand how data intensive analytical techniques such as metabolomics can be used to identify unique features and profiles associated with different stages of human decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that metabolomics is an innovative tool capable of advancing the understanding of human decomposition rates by monitoring the metabolic changes in a variety of matrices (soil, decomposition fluids, and insect larvae) associated with human cadavers.

Recent advances in Liquid Chromatography/Mass Spectrometry (LC/MS) have allowed metabolomics to be a viable approach for monitoring dynamic biological processes, including those relevant to forensic science. Not only can LC/MS-based metabolomics discover biomarkers for specific metabolic processes, the technique also detects thousands of molecules in a single analysis that can inform about the global metabolic state of the system.

Human donors with no outward trauma were placed on the soil surface at the University of Tennessee Anthropology Research Facility (ARF) and allowed to naturally decompose. Soil samples from the Cadaver Decomposition Island (CDI), as well as control soils from outside the CDI, were collected at regular intervals tied to temperature and time (Accumulated Degree Hours [ADH]). When present, decomposition fluid and larval samples were collected until cadavers completed active decay, as determined by cessation of purged decomposition fluid from the trunk.

Each collected sample was flash frozen in liquid nitrogen and stored at -80°C until extraction. Soil samples from a single time point were homogenized prior to freezing. Decomposition fluids were collected using sterile syringes when visibly pooling on the soil surface. Insect larvae masses were collected at representative development stages, and care was taken to ensure that enough individuals would remain to avoid disrupting the colony. Metabolites were extracted from all matrices using modified versions of known methods,<sup>1</sup> and analyses were performed using an Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) by adapting the method from Lu et. al,<sup>1</sup> onto an Exactive™ Plus Orbitrap™ Mass Spectrometer.

While metabolomics has emerged as a powerful analytical tool for increasingly larger studies, problems have arisen during the data processing and analysis stages, as most of the common processing programs and workflows require batching samples to manage the computational efforts. However, using batches to analyze metabolomics data sets can also introduce problems when comparing data among samples due to computational variations in peak detection and alignment both within and between batches.<sup>2,3</sup> This is especially relevant for studies conducted over multiple years, such as this project, and attempts to overcome this issue and build a database of metabolite features associated with stages of human decomposition will be discussed.

Samples collected from 14 donors yielded >3,000 samples subjected to metabolomic analysis. Samples were placed in batches at random for extraction and analysis; however, replicate samples were analyzed on the same day. The number of injections in each batch (day) averaged around 63, and in total 58 batches have been analyzed. Results from these samples yielded several thousand spectral features between all matrices. Of these features, 2%–5% have been identified based on an in-house library of ~450 metabolites.

The known metabolite list from the Quality Control (QC) samples were used to identify a set of metabolites conserved across the majority of samples, which were then used to perform the alignment across all batches. Once all samples were properly aligned, all features were subjected to standard statistical analysis, specifically Partial Least Squares Discriminant Analysis (PLSDA) to identify features (or a set of features) unique to each sample, matrix, or donor. Further machine learning algorithms using only the list of known metabolites from soil samples identified several metabolites highly correlated with either the decomposition or control soil. Specifically, the random forest algorithm predicted control and decomposition samples with 93% accuracy in a test sample, which was not used in the model fitting process, and chose pantothenate, creatine, taurine, xylitol, xanthosine, and hypoxanthine as important indicators for classifying decomposition soils.<sup>4</sup> Further work is in progress to incorporate the unknown features and the other matrices into these analyses as well as correlation to the decomposition time course using ADH.

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### Metabolomics, Forensic Chemistry, Machine Learning

## A97 A New Quantitative Method for Analyzing Burned Human Remains

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**Learning Overview:** After attending this presentation, attendees will better understand the variation in thermal alterations on human remains and variables that can contribute to these heat-related changes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for a more quantifiable approach to analyzing burned human remains that can aid investigators in building a legal case.

Fatal fires produce a range of physical alterations to the body, from blistering of soft tissue to the calcination of bones.<sup>1,2</sup> These physical alterations leave patterns that can be studied and analyzed to interpret perimortem events. Many anthropological-based classification systems progress quickly through the burn process, capturing only the advanced stages of heat-related damage and therefore are not widely used today. Previous models are primarily descriptive, with little to no attempt at quantifying the amount of thermal damage on a human body.<sup>3-5</sup> There is a need to develop a quantitative method that encompasses all thermal alterations and can be more broadly applied to a range of fire environments.

This study involves observational experiments of the burning of 90 donated human cadavers. Data were collected as part of the Fatal Fire Death Investigation Course by the San Luis Obispo Fire Investigation Strike Team, Inc. Cadavers were placed in vehicles, structures, confined spaces, and outdoor fire contexts. All physical alterations to both soft and skeletal tissues were documented with digital photography. Temperature data were collected through the use of thermal couples and thermal imaging devices placed on multiple locations and depths directly on the human remains. The new model was developed based on observations of thermal changes noted during these experiments. This model assesses burned remains by applying a Total Body Score (TBS) based on the affected body region (i.e., skull, upper and lower limbs, thorax, and hands/feet). Once the visual analysis was completed, all scores for each region were added together to form a TBS and compared across all fire environments to identify any patterns.

This study found notable differences in TBS between fire environments. Outdoor and confined-space fires exhibited the highest degrees of fragmentation and calcination across all bodily regions, which is consistent with some of the highest scores observed (TBS scores between 28–33). There were notable differences between compartment and trunk fire individuals. Compartment individuals exhibited widespread charring and calcination to the skull, hands, and feet, and limited soft tissue loss on all other bodily regions, which resulted in significantly lower scores (TBS scores between 19–26). Trunk individuals exhibited widespread calcination and fragmentation to the hands, feet, limbs, and skull (TBS score between 27–33), which is consistent with some of the highest scores recorded. Structure fire individuals exhibited limited bone exposure and soft tissue loss across all bodily regions, which resulted in some of the lowest scores observed (TBS scores between 8–20). The results of this study demonstrate TBS varied based on time, temperature, and ventilation patterns within each fire environment, illustrating a relationship between these variables. This study demonstrates the applicability of this model to a variety of fire conditions. More importantly, it demonstrates there is a pattern between fire environment and bodily conditions, making it possible to model heat-related damage. As data collection progresses, these TBS will be taken to form a more robust model for estimating exposure time to *in situ* conditions.

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### Burned Human Remains, Classification System, Forensic Anthropology

## A98 A Refined Classification System for Thermally Damaged Human Remains by Body Segment

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**Learning Overview:** The goal of this presentation is to provide a comprehensive regional approach to the classification of thermal damage of soft tissue and bone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of how to apply a classification that encompasses soft tissue changes, body movement during fire, and skeletal damage to allow categorization of damage by segment. While minor discrepancies by segment are normal, significant differences by body segment will require explanation in terms of fire dynamics, suppression efforts and/or protection applicable to certain body segments.

Previously, a whole-body classification system was presented. Here, this presentation has refined this system and created a regional approach that provides flexibility while simultaneously providing a realistic and easily reproducible assessment of the scale and damage associated with burning. This presentation outlines a six-stage system in which the body may be assessed in segments consisting of the head, torso, arms, and legs. The progression of changes associated with each stage is generated from over 12 years of experience with the San Luis Obispo Fire Investigation Strike Team (SLO FIST).

Classification of burning has normally utilized the Crow-Glassman Scale (CGS), developed from assessment of end-product burning. The CGS scale has five phases: (1) singed and blistered body, (2) charred body, (3) body with loss of arms and/or legs but head present, (4) partial body with significant limb and skull loss, and (5) cremated. While helpful, this system does not allow for assessment by body segment and thus the flexibility to address differential burning and is primarily focused on the product as found by the anthropologist. It does not discuss changes in body position due to the heat.

At the SLO FIST Forensic Fire Death Investigation Course (FFDIC), training for law enforcement, fire investigators, and coroners utilizes donated human remains in realistic fire scenarios. This training is combined with scientific research on body destruction, fire progression, and temperature-dependent changes, based on thermocouple data from the remains and the scenario. Fires are suppressed at various degrees of burning in order to meet the needs of the individual scenario. Consequently, bodies are examined across the spectrum of burn damage in sufficient numbers to provide a good sample size.

Utilizing photographs, video, and recorded statements, this study constructed a classification system for fire damage. Each of the six stages represents a range and is bracketed from early to advanced phases. This range is shown with images and descriptions, allowing assignment to an “a (early)” or “b (advanced)” phase of the stage. Assessment information focuses not only on the overall description of the remains at each stage but on the features that define the transition from one stage to the next and from early to advanced phases. Rather than depend on photographs, where ability to discern the critical features is difficult, the classification system uses inked illustrations with written descriptions.

Significant differences in the assessment scores within a single body may highlight where additional explanation is required. For example, a person on an armchair may have much greater damage to the legs while the torso, arms, and head are significantly less burned as the substrate collapsed, tipping the body away from the central focus of the flames.

While assessment of photographs is possible, the system works far better when used directly on the burned remains. The system is also designed to be utilized by many in the death investigation field and can be used in the field and/or in the laboratory/morgue. Worksheets for recording burn damage have also been developed and facilitate recording on soft tissue, bones, and dentition.

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### Thermal Damage, Burned Human Bone, Fire Death

## A99 Reporting Biases Between Missing Persons and Unidentified Persons in the United States

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**Learning Overview:** After attending this presentation, attendees will be aware of potential bias associated with reporting in the National Missing and Unidentified Persons System (NamUs). Discrepancies between Missing Persons (MP) and Unidentified Persons (UP) datasets will be explored using the qualitative biological variables of ancestry and sex.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying levels of inherent bias in sex and ancestry via case data to stimulate discussion of current labeling systems and forensic anthropological methodology in the United States.

The NamUS database contains data submitted across the United States. The database contains MPs and UPs—these two datasets may not be synonymous for a variety of reasons (e.g., reporting biases, method inaccuracies). However, the datasets *should* closely approximate one another unless biases are present in forensic methodology (UP data) or in the reporting culture (MP data). The purpose of this project is to understand if any patterns of biases exist.

NamUs case report data were obtained for 10,000 random UPs and 10,000 random MPs. Individuals with one recorded ancestry (American Indian/Alaska Native, Asian, Black, Hawaiian/Pacific Islander, Hispanic/Latino, White) and sex (female, male) category were chosen to minimize variables and decrease computational complexity. The final sample includes MP ( $n = 9,079$ ) and UP ( $n = 6,760$ ) data. Many individuals self-identify socially to multiple ethnicities/ancestral groups and/or to trans/non-binary genders, classifications not easily recorded in the current NamUS system. It is acknowledged that single ancestry and binomial sex categories are unrealistic conventions, but they are applicable for this preliminary theoretical exploration. Counts by ancestry and sex were cross tabulated and converted to proportions to control for sample size. The MP and UP datasets were compared to each other using chi-square tests. These comparisons were done for sex, ancestry, and the interaction of both variables.

Assuming homogeneity, one would expect a near 1:1 ratio between MPs and UPs. However, the chi-square test indicated statistically significant differences between these overall datasets ( $p = 0.003$ ). Females were significantly underrepresented in the UP records compared to males ( $p = 0.002$ ). For ancestry, American Indian/Alaska Native ( $p = 0.004$ ), Hawaiian/Pacific Islander ( $p = 0.001$ ), and Hispanic/Latino ( $p = 0.074$ ) were all significantly different, indicating underrepresentation among the UPs when compared to the MP samples. When combining sex and ancestry, only two groups showed significant differences: Hispanic/Latino males ( $p = 0.010$ ) were overrepresented in UPs compared to MPs, while the opposite was true for White females ( $p = 0.014$ ).

These results suggest several factors may influence bias in reporting (MP) or methodology (UP). For ancestry, differences may reflect biases in one or several of the following: (1) a reliance on the three-group model because practitioners are either unfamiliar with refined methodology or uncomfortable using them in practice; (2) broad category estimates when reporting for fear of inaccuracy; and/or (3) the absence of reference data and/or sampling bias. Examining only sex, this study found females were underrepresented in the UP data compared to MPs, indicating a potential sex bias in forensic methodology toward male classification, contrary to published literature.<sup>1</sup> White women are the only group significantly underrepresented in the MP data when combining sex/ancestry. These data show a bias against UP White females, but not for UP and MP minority females. Theoretically, Missing White Woman Syndrome assumes White women are reported missing over other socially constructed races and genders.<sup>2,3</sup> Therefore, White females should be the most accurate representation using forensic methods. Additionally, there is a well-documented phenomenon of underreporting for minority females (MP), but this study may be under-classifying minority females with these methods (UP). These data also suggest underreporting for Hispanic males. The most parsimonious explanation points to bias in sex and/or ancestry methodology for White women, underreporting of Hispanic males, and likely underreporting minority women.

This research provides evidence for biases both in reporting and forensic anthropological methodology related to sex and ancestry estimation. The call for population-specific methods for sex and ancestry represent one way forward to potentially minimize these biases.

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3. Moss J.L. The forgotten victims of missing white woman syndrome: An examination of legal measures that contribute to the lack of search and recovery of missing black girls and women. *William & Mary Journal of Race, Gender, and Social Justice* 2019;25(3), 737-764.

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### Forensic Anthropology, Biological Profile, NamUs



## A100 America Since Wounded Knee: Contextual Vulnerabilities in the Ongoing Missing and Murdered Indigenous Women (MMIW) Crisis

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**Learning Overview:** After attending this presentation, attendees will begin to understand some of the compounding societal circumstances impacting communities designated as hotspots for the MMIW crisis in the United States. Through a deeper, more localized approach to understanding this crisis, forensic practitioners will gain knowledge relating to societal trends that often lead to higher MMIW rates, certain factors to take into consideration when working on MMIW-related cases, as well as gaining a general understanding of the crisis at large and current efforts being made to help mitigate the crisis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness about the ongoing MMIW crisis through focusing on the differential societal structures involved, local tensions between populations, and community-based efforts to help indigenous populations.

For centuries, indigenous women have faced ongoing genocidal actions, attempted slavery, many thefts of land through forced relocations, disease warfare, attempted cultural annihilation, the kidnapping, abuse, and death of their children, famine, poverty, violence, and racism, among other things. While some may like to view these tragedies as having occurred only in the past, many of these practices continue to represent the reality facing many indigenous women across the United States today. Previous research conducted for this study revealed a total of 23 locations determined to be hotspots for MMIW cases in the United States.

The purpose of the present study has been to investigate a broad range of shared societal factors for the 23 MMIW hotspots in the United States in order to identify any specific trends that may contribute to the perpetuation of the MMIW crisis. Across the 23 hotspot locations, several factors were determined to be significantly ( $p < 0.001$ ) higher than the national average, including: the total number of Native Americans living there, the number of individuals who do not have health insurance, the rate of poverty, the rate of homelessness, the violent crime rate, the property crime rate, and the number of individuals with substance abuse problems (i.e., alcohol, marijuana, and cocaine). Other factors present across the 23 hotspots that likely contribute to the MMIW crisis include: human trafficking, racial tensions, each location's proximity to military bases, major highways, and resource extraction sites (i.e., hydraulic fracking, oil drilling, coal mining).

The massacre of Native Americans at Wounded Knee by the United States Army in 1890 is known for being the last massacre against indigenous people in the United States; however, the ongoing MMIW crisis could be described as merely a more silent type of massacre. Indigenous people are one of the most vulnerable racial demographics in the United States presently, due to the lasting effects of over 500 years of colonization, oppression, systemic discrimination, ethnic cleansing, and genocide. By situating this crisis in the roots of oppression and systemic discrimination in the United States, we capture a more complex view of the particular needs and vulnerabilities of indigenous communities.

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**MMIW Crisis, Racial Violence, Homicide**



## A101 Structural Vulnerability in Transgender and Non-Binary Decedent Populations: Analytical Considerations and Harm-Reduction Strategies

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**Learning Overview:** After attending this presentation, attendees will have learned about structural vulnerabilities affecting transgender and non-binary individuals, how these vulnerabilities may affect the circumstances and investigations of their deaths, and how a structural vulnerability assessment can reduce harm in the identification process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework for understanding barriers to identification and offering suggestions for harm reduction in forensic cases involving potential transgender and non-binary decedents.

Structural vulnerability assessments have been proposed as a way to plan ethical and equitable treatment in medicine and research.<sup>1</sup> Soler et al. propose the application of a structural vulnerability assessment to forensic work.<sup>2</sup> They explore vulnerabilities specific to undocumented migrants and argue that understanding these vulnerabilities is essential to harm reduction in investigation and identification efforts. While different intersecting socioeconomic and cultural factors affect the life and death experiences of transgender populations, this study proposes that a structural vulnerability assessment model is also a useful lens for navigating non-binary cases.

The physical bodies of transgender decedents may record the biological consequences of structural vulnerability. Disparities can manifest skeletally as antemortem/perimortem trauma and non-specific indicators of stress and in the treatment of bodies after death. Applying the temporal framework proposed by Soler et al., antemortem, perimortem, and postmortem vulnerabilities will be explored for non-binary decedents.<sup>2</sup>

**Antemortem:** During life, systemic marginalization can result in economic (e.g., low wages, barriers to education and employment, unsafe working conditions), social (e.g., disintegrating ties with family support networks, susceptibility to interpersonal violence), and health (poor access to food and housing resources, refusal of care by providers) disparities. Many issues affecting transgender and non-binary people are intersectional; Black, Indigenous, and low-income decedents may also be affected by these issues.

**Perimortem:** Transgender and non-binary people face increased risk of violent death and suicide, so they may become subjects of medicolegal investigations at a disproportionate rate compared to their share of the population.<sup>3</sup> Simultaneously, gender identity or status may not be accurately recognized in the initial stages of a death investigation.

**Postmortem:** Postmortem identification efforts often uncover an individual's "deadname," rather than the name by which they lived and were known to friends and chosen family. An unsupportive biological family or reporting agency may misgender or deadname decedents in reports, thus impeding search efforts. Some families may also refuse to initiate a search or to collect remains once identified.

Assessment of trans-specific structural vulnerabilities may inform investigative strategies and identification efforts and ensure these efforts prevent further harm to the decedent, their memory, and their family (biological or chosen). Forensic anthropologists can participate in harm reduction by: (1) acknowledging that the existing binary (Jane or John) for Does does not encapsulate lived experiences of many decedents; (2) recognizing that biological and contextual evidence is necessary to achieve identification; and (3) appreciating that distributing this information may cause further harm.

Just as there is no one way to "be" transgender or non-binary in life, there is no one presentation in death. Anthropologists should recognize and accept the "gray areas" in identifying and researching transgender and non-binary decedents, while generating inclusive and accurate language in reports and communications with law enforcement. Sex estimation is a critical piece of the biological profile but has limitations. This study cautions that research centered on skeletal indicators of gender identity poses ethical concerns because medicalization of gender has been used historically to justify non-consensual medical intervention.

Anthropologists should ask for and accept assistance from trans-informed community members and advocates during the investigation process, as detailed interpretation of remains, associated clothing/artifacts, and recovery context is necessary in cases with scant evidence or long postmortem intervals. Collaborative approaches between forensic anthropologists and other professionals (e.g., genealogists, LGBTQ+ advocates) can result in sensitive and accurate identifications for individuals whose physical appearance at death may not correspond to their government-issued records.<sup>4</sup>

Finally, while a principal tenet of forensic anthropology is to return the dead to their family, it is emphasized here that this process may cause further harm. Honoring lived identity after death is a complex process; while community members do this as an extension of mourning the dead, forensic practitioners have only recently begun to explore these complexities.

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### Transgender, Sex Estimation, Identification

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## A102 Forensic Anthropology is WEIRD: Knowledge Production by Whom for Whom

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**Learning Overview:** After attending this presentation, attendees will understand the current state of forensic anthropology research regarding the demographics of sample selection and knowledge production.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the underrepresentation of certain global samples in recent forensic anthropological research and the discrepancy in impact between Western, Educated, Industrialized, Rich, and Democratic (WEIRD) and non-WEIRD publications. In addition, this research calls for better representation of samples and greater global collaboration.

Most forensic anthropologists and the populations they study are WEIRD. In their interventions in biological anthropology, Clancy and Davis contend that WEIRD, a euphemism for white (Western European-derived populations) both as scientists and subjects, skew the predominating narrative of the human condition.<sup>1</sup> How their framework specifically applies to forensic anthropology would benefit from further rumination. The researchers argue that the scientific enterprise of forensic anthropology is a WEIRD space unique within biological anthropology in that it: (1) is touted as an applied, practical, and “objective” tool that must operate within medicolegal systems; (2) is a subspecialty with board certification and accreditation standards; and (3) holds ancestry and race as core to its practice.

A bibliometric survey of publications over the past five years in the most relevant journals to the field yielded 793 articles. Information was collected from each article, including article type, category, authors and location, samples, and times cited. Articles originated from six academic journals: *American Journal of Physical Anthropology*, *Forensic Anthropology*, *Forensic Science International*, *International Journal of Legal Medicine*, *Journal of Forensic Sciences*, and *Science & Justice*. Summary statistics were generated from the data to evaluate proportional differences in publications and samples of WEIRD and non-WEIRD groups. In addition, citation *h*-index values were calculated to evaluate the relative impact of each country’s publications on the field of forensic anthropology.

Results from summary statistics found that 73% of authors originate from WEIRD contexts. In papers specifically studying ancestry, European-derived populations are included 81% of the time as a category for comparison to other groups, while only 12% do not include Europeans in their comparisons, reinforcing a historic tendency to measure all human variation against one particular norm (i.e., White or European). Furthermore, 49% of papers where ancestry estimation is not the main focus use White subjects solely or in part. Papers authored from WEIRD contexts receive significantly more citations, more than two times, than non-WEIRD counterparts. Differences in the *h*-index of countries show that the United States surpasses all other countries in research output by a large margin ( $\Delta h \sim 2,000$ ), followed by other WEIRD countries such as the United Kingdom, Italy, and France.

This bibliometric study demonstrates the extensive nature of knowledge production of WEIRD contexts in forensic anthropology. By highlighting the gaps and underrepresentation of non-WEIRD research and samples, forensic anthropologists can begin to actively consider collaboration and new ways to achieve greater representation.

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### WEIRD, Ancestry, Knowledge Production

## A103 Terminology Used to Describe Human Variation in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will better understand the terminology that has been used to describe skeletal human variation in the *Journal of Forensic Sciences (JFS)* between 1972 and 2020.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying terminology to understand temporal trends in forensic anthropological research on human variation and how this terminology is relevant to resolving missing persons cases.

Biological anthropology has long studied human variation over time and in extant populations. In forensic anthropology, this work has included estimations of what is now typically called “ancestry.” This work has been subjected to criticism from other scholars within anthropology, but has been defended as being necessary to resolve missing persons cases. The goal of this research is to examine the use of terminology within forensic anthropology over time and align that with data on missing persons and the United States census to evaluate the importance of that data within a medicolegal context.

To evaluate terminology usage over time, this study evaluated articles in the *JFS* from 1972 to 2020 ( $n=11,305$ ). Within these articles, this study identified those that used any relevant term (e.g., race, ancestry, White, Black;  $n=836$ ), and further winnowed the data to only those that were of anthropological interest ( $n=360$ ). Terminology was placed into two categories: classifiers and descriptors. Classifiers include the term authors used to describe their broad categories. These were standardized into one of five options: “race,” “ancestry,” “population,” “ethnic,” or “other.” Descriptors included terms used to describe individuals within these classificatory terms. Descriptors were standardized in terms of word order, capitalization, and hyphenation (e.g., African-American and African American, or American White and White American). These data were also compared with terminology and data kept by the National Missing and Unidentified Persons System (NamUs) as well as the United States census to understand how terms in forensic anthropology are relevant to resolving missing persons cases.

For classifiers, “race” was the most prevalent, found in 42.4% of articles, followed by “ancestry” (30.6%), “population” (14.6%), “ethnic” (7.6%), and “other” (4.8%). A total of 160 distinct descriptors were identified, 98 of which appear only once in the articles analyzed (e.g., “mixed race,” “trihybrid,” “Latino”), many of which are considered offensive terms to use. Of the remaining 62 that were used in at least two different articles, the most commonly used descriptors (>2%) were “White” (16.0%), “Black” (13.8%), “American Black” (5.5%), “American White” (5.3%), “European” (4.5%), “Hispanic” (3.0%), “Native American” (2.9%), “Caucasian” (2.7%), “African” (2.6%), “African American” (2.4%), “American Indian” (2.1%), and “Asian” (2.2). Temporal trends show a peak in these types of articles in the 1990s and mid-2000s. While “race” was used early on, the terms “ancestry” and “population” have become more common in the past 30 years.

The NamUs database does not use the same terminology seen in *JFS*, illustrating a disconnect in the terms used to identify the missing and unidentified with those used in anthropological research. There are also biases in the data of the missing in terms of age and sex that are not echoed in the data of the unidentified, which could indicate deficiencies in these estimations of the biological profile. There is a bias in terms of race/ethnicity of the missing and unidentified and the United States census data. While “White” individuals make up 72.4% of the United States population, they only constitute 50.3% of the missing and 29.4% of the unidentified. “Black/African Americans” comprise 12.6% of the population and make up 18.6% of the missing and 13.9% of the unidentified.

In this review, it is clear that there are differences in the terminology used in forensic anthropology and that used in the census and missing-persons reports. Further, refining methods for sex and age estimation may also help significantly in resolving missing persons cases, as illustrated in the NamUs data. It is important for forensic anthropologists to be cognizant of the terms they use in medicolegal contexts, publications, and in public and/or professional spaces. The continued use of misrepresentative and improper language further marginalizes groups and perpetuates oppression rooted in systemic racism. Acknowledgement of prior wrongs and proper use of terminology will serve to improve our science and discipline.

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### Ancestry, Race, Language

## A104 Assumed Differences and Unquestioned Typologies: The Oversimplification of Race and Ancestry in Forensic Anthropology

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**Learning Overview:** After attending this presentation, attendees will better understand how forensic anthropologists have used and defined “race” and “ancestry” in research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropologists have failed to thoroughly define concepts of “race” and “ancestry,” resulting in an oversimplification of complex biocultural processes. Additionally, forensic anthropologists have inconsistently explored the reasons for group-level differences or similarities, including population histories and/or structures and microevolutionary processes.

The discipline of forensic anthropology, a field tasked with analyzing skeletal remains to enable personal identification, has long assessed “race” or “ancestry” as part of the biological profile. Early methodological approaches were strictly typological in nature, which enabled their incorporation into racist perspectives. While practitioners may have shifted terminology from “race” to “ancestry” to describe regionally patterned human skeletal variation, the degree to which they have changed or critiqued long-embedded typological approaches remains unclear. This lack of clarity is problematic as it places the onus on the consumers of the literature to deduce what is meant by “race” or “ancestry” and leads to the faulty assumption that all researchers share the same, or similar, working definitions of “race” and “ancestry.”

Thus, this study reviewed 118 peer-reviewed forensic anthropology articles published between 1966 and 2020 in the *Journal of American Physical Anthropology*, *Journal of Forensic Sciences*, *Forensic Science International*, and *Forensic Anthropology* by identifying any combination of the publications’ key words “race,” “ancestry,” “ethnicity,” or “population affinity.” Data collected for each article included the publication year, methods utilized, groups studied, terminology used, and whether the authors defined “race” and “ancestry.” Additionally, it was noted which human groups were analyzed, the terminology utilized to identify the groups, whether the authors critiqued or otherwise contextualized the use of their chosen approaches, and whether they provided biocultural explanations for the observed population differences or similarities.

The data supported the primary hypothesis that, while the term “ancestry” has supplanted the term “race” to describe affinities with human populations, this change in terminology has not brought concurrent changes in approach, nor deeper scrutiny of underlying concepts. In this sample, the key terms “race” and “ancestry” were infrequently defined (in 14% and 11% of articles, respectively), and a plethora of social, geographic, and pseudo-scientific terms persisted in reference to human population groups—including, as recently as 2015, the “Caucasoid, Mongoloid, Negroid” scheme. Forensic anthropologists have increasingly engaged with questions addressing the biocultural forces patterning human biological variation: 64% of studies postdating 1999 discussed population histories, population structures, and microevolution, compared with 38% of studies published between 1966 and 1999. However, the amount of attention given to population histories, populations structures, and microevolutionary processes seemed to differ depending on the groups under study and their perceived “complexity.” Population histories and structures were often limited to these “complex” groups, such as Hispanics, South Africans, or Asian-derived populations. Further, fewer studies contextualized or critiqued approaches to analyzing population variation (32% of studies postdating 1999, compared with 4% from 1966–1999), and virtually no studies considered the possibility that skeletal variation reflected embodied social inequality (5% of studies postdating 1999; 0% from 1966–1999).

Complex concepts demand complex approaches, particularly when they carry a history of injustice and oppression and have contributed to racist ideologies. Thus, it is imperative that forensic anthropologists explicitly define the human population concepts that structure and guide their research. While the lack of explicit definitions regarding “race” and “ancestry” and the limited engagement with the biocultural processes that contribute to skeletal variability in the published literature may be partly due to editorial and peer-review pressures, it is likely that many forensic anthropologists have not interrogated their own perspectives and definitions. This lack of interrogation and clarity contributes to the faulty notion that all forensic anthropologists share similar definitions and leads to an oversimplification of complex biocultural processes.

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### Forensic Anthropology, Human Biological Variation, Critical Race Theory

## A105 Forensic Science and the Living: The Ethics of “Determining” Age From Minor Children

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**Learning Overview:** After attending this presentation, attendees will better understand what the ethical issues are surrounding the application of forensic anthropological and odontological methods using bone and tooth development to assign a chronological age to a living person for the purposes of determining whether they have reached the legal age of 18.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and the performance of attendees moving forward as they consider whether or not to conduct cases for law enforcement that involve age determinations of living people, as the punitive consequences for such persons can be severe and methodological accuracy varies substantially.

Adverse sociopolitical developments in the global south have led to a rising number of fleeing citizens seeking refuge in more stable countries. Of the 744,000 individuals who applied for first-time asylum within European Union states in 2019, nearly 27% were under the age of 18. Once faced with an immigration official in the receiving country, there are questions to which they must respond. One of these is chronological age, important because minor children have different protections and rights than do adults (18+ years). Unaccompanied minor children are considered especially vulnerable, and in the United States, are afforded special protections by the Flores Settlement and the Trafficking Victims Protection Reauthorization Act (TVPRA). In some cases, there are questions surrounding the minor’s purported age: either the documents they have brought with them cannot be verified, or they are non-existent. When officials believe a migrant claiming to be a minor is an adult, the onus is on the government to prove this—which, according to the TVPRA, they must primarily do by reviewing documents and conducting interviews. Some element of medical age evaluation is allowed, via examination of skeletal or dental markers of growth and development to estimate biological age.

When considering the application of both skeletal and dental age estimation techniques to living children, a false equivalency between chronological and biological age is assumed. Genetics, nutritional status, infectious disease exposure, and psycho-social and intergenerational stress have all been shown to affect growth and development patterns. Therefore, the lack of population-specific developmental references for unaccompanied minors born in the global south renders standard developmental references inapplicable. Further, tests of skeletal aging techniques based on European-American children, such as the Greulich-Pyle wrist method, have lower-than-acceptable accuracy for numerous populations. Similarly, dental developmental data are predominantly drawn from European-American and African-American populations whose early-life experiences are dissimilar from those in the global south. The third molar (most often examined in dental age estimations as it is the last tooth to complete development) is the most variable of all dentition; with some individuals completing root development as early as 13 years or as late as 19–20 years. Critically, it is impossible to test the accuracy of skeletal and dental age estimates among unaccompanied minors for whom chronological age is unknown. Forensic practitioners are therefore unable to assess the degree of error associated with their estimates, despite the fact that outcomes of these evaluations have critical legal implications.

Ethical issues surrounding the application of medical age estimation methods include whether voluntary consent can truly be given in circumstances related to asylum seeking, bias against individuals from countries without standardized birth registration systems, psychological (re)traumatization of minor children, and unnecessary exposure to radiation and medical testing. An increasing number of European medical and forensic professionals are taking strong stances against participation in medical age evaluations, even as the 2018 European Union Joint Research Center’s Report on Medical Age Assessment of Juvenile Migrants suggests these methods are appropriate and protective against the arbitrary nature of more intuitive age assessment methods. As anthropologists, we are uniquely qualified to understand both the scientific impact of our participation in living age assessment as well as the profound socio-cultural factors impacting the lives and experiences of migrant children. Forensic practitioners may believe they are assisting in humanitarian efforts when providing age assessments, but when scientific methods are misapplied and ethical factors are not thoroughly considered, participation in medical age assessments can cause significant harm to migrant children.

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### Age Determination, Ethics, Migrants



## **A106 Forensic Humanitarian Ethics in a Domestic Medicolegal Context**

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**WITHDRAWN**

## A107 *Bakeng Se Afrika* and the Ethics of Digital Repositories of Human Remains

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge on the European co-funded project, *Bakeng se Afrika*—a digital skeletal repository of South African individuals housed in South Africa. This presentation will explore the gaps in the ethics surrounding data sharing and ownership of digital copies of a human skeleton.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to knowledge on current issues pertaining to the sharing of digital copies of a human skeleton.

The purpose of this presentation is to discuss whole-body consent and ownership of digital skeletal data in South Africa and its impact on dissemination of digital skeletal repositories. In South Africa, the use of human biological material and associated personal information for research/teaching is addressed in the National Health Act 61, 2003, Protection of Personal Information Act (POPIA) 4 2013, and material transfer agreements between institutions.<sup>1-3</sup> Whole body donations to medical schools are given under “broad consent.”<sup>1</sup> Broad consent means all soft and hard tissues of that person can be used for any current and future research. Medical schools, as Higher Education Institutions, claim ownership of the human material and can grant access to it based on approval from institutional ethics review boards. The provenance of whole-body donations is from two sources: willful donated and unclaimed persons. If a person dies in a government hospital and is not claimed within 30 days of their death, the Director General donates their body to the nearest medical school. While an unclaimed body is legally donated to a medical school in South Africa, anatomists are currently debating the ethics regarding using the physical remains of unclaimed persons for research and teaching, despite upholding their anonymity.

Methods for conducting research/teaching have expanded since the creation of the current legislation for the legal and ethical use of human remains. Although human skeletal repositories are composed of donated individuals (either via themselves, their next of kin, or the government), the advancement of technology has given rise to gray areas in the associated ethics and the meaning of “consent,” particularly with digital images of human bone. The digital images of a human skeleton are not legally ascribed as *symbolizing* the person, so the current ethics/issues guidelines do not necessarily apply, leaving an opening for amendment but also possible abuse. The gray areas of legislation in South Africa provide the consortium an opportunity to create ethical guidelines for the treatment of digital imaging within a diverse socio-cultural and socio-political landscape. Some areas for investigation include issues of consent, personification of “life” in bone, and ownership/Intellectual Property (IP) of 3D reconstructions and prints of a skeleton.

Digital human skeletal repositories are increasing globally. With digital imaging, researchers can easily and cost effectively obtain and collaborate on projects involving human skeletons. An example of such a project is *Bakeng se Afrika*. *Bakeng se Afrika* (“for Africa”) is an Erasmus+ European Union-funded capacity building project in higher education between Europe and South Africa, with the purpose of developing a digital data repository of human skeletal remains, both living and deceased, of South Africans for research and education. The research consortium involves three South African Higher Education Institutions (HEIs), namely the University of Pretoria, Stellenbosch University and Sefako Magatho University, and the Nuclear Energy Corporation of South Africa (NECSA), along with four European HEI’s, the University of Bordeaux, Centre National de la Recherche Scientifique (CNRS), University of Coimbra, and the Catholic University of Leuven.

Advancements in digital imaging technology provide an opportunity for the global application of biological anthropology as a discipline. Data sharing is aligned with building capacity in research/education in biological anthropology in developing countries, attracting high-quality and diverse postgraduate students, improving success in international research grants, validating research methodology, and applying outcomes to both medicolegal and health-related fields. Yet who stands to benefit from this advancement in data acquisition and research outcomes—the donors, the researchers, or the society in which the data are contextualized?

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3. Thaldar D.W., Botes M., Nienaber A. South Africa’s new Standard Material Transfer Agreement: proposals for improvements and pointers for implementation. *BMC Medical Ethics* 21 (85) (2020)

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### Micro-CT Scans, 3D Prints, Digital Data Sharing

## A108 The Ethics of Combatants as Investigators in the Search for People Missing Due to Armed Conflict

*Derek Congram, PhD\*, University of Toronto, Toronto, ON M5S 0A7, CANADA; Maria L. Urbano, FARC, Bogota, COLOMBIA; Tilzia Quintero, FARC, Bogota, COLOMBIA; Jhon L. Gonzalez, BA, FARC, Bogota, COLOMBIA; Jaime Parra, FARC, Bogota, COLOMBIA*

**Learning Overview:** This presentation will explore the usefulness of incorporating former combatants in the search for those who had disappeared during armed conflict. Attendees will learn about the unique types of information that those who have lived with armed conflict first-hand—possibly direct witnesses of disappearances—bring to investigations. Additionally, attendees will learn how this approach compares to searches that normally involve certain technical forensic expertise, but which are not contextualized and not informed by all of the geographic and sociopolitical aspects that have an impact on where the disappeared will be found.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating in very practical as well as ethical terms the implications of not only the participation but also the active protagonism of ex-combatants in this type of search. In this presentation, it will be argued that their active participation assists with the location of disappeared persons, but also serves as a form of recognition and reconciliation, with a potentially profound reparative impact in the frame of restorative justice.

Often forensic anthropologists begin their work when human remains are discovered by chance. In other situations, this begins with a search for a clandestine grave or the remains of a disappeared person, presumed dead. Although there are many high-tech archaeological tools to aid in searches, the most common approach is to rely on oral testimony. Obviously, direct witnesses are the most useful.

Although there are those who would argue that the inclusion of people, possibly responsible for disappearance, for the search for the missing implies an ethical compromise, this perspective reduces the identification of those involved in conflict to an overly simplistic duality of perpetrators and victims. As well, it fails to recognize the importance of identities (e.g., combatants and non-combatants) in acts of disappearances and the subsequent searches.

In November 2017, a pilot training project was held on the investigation of missing persons in Colombia. The supposed beneficiaries of the training were members of the Fuerzas Armadas Revolucionarias de Colombia—Ejército del Pueblo (FARC-EP) or Revolutionary Armed Forces of Colombia—People’s Army, the guerrilla group that signed a Peace Agreement with the Colombian government after more than 50 years of armed conflict. At the end of a week of lectures and practical exercises, one of the leaders of the FARC stood up to provide feedback: “You,” he said, referring to the trainers, medicolegal experts, and delegates of an international humanitarian organization, “have a lot of experience in filling out forms, conducting formal interviews, etc. But what *you* must recognize is that *we* have, for decades, been looking for our own missing.” With this comment, the trainees made clear their capacity to be the trainers.

The recent experience in Colombia of the search for missing persons is an unprecedented experiment. Ex-combatants—responsible for and victims of disappearance—now have also become investigators of the disappeared. The experiment has produced very interesting results: to date more than 320 documented cases have been handed over to the authorities, and they have provided information leading to the recovery from clandestine graves of the remains of many other people around the country. The key to this process has been an extrajudicial and humanitarian mechanism, a product of negotiations that lead to a peace agreement. The newly resolved cases are those that the state had been unable to resolve, in some cases for decades following the disappearances.

This presentation is a product of the work conducted by the investigations that has included forensic anthropologists and ex-combatants: several rounds of capacity building, the exchange of information, witness interviews, grave inspections (both successful and failed), and subsequent analysis of the results to identify shortcomings and gains in the process.

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### Disappeared Persons, Ethics, Armed Conflict





## **A109 Proxies for Race: The Disconnect of Forensic Anthropology From Anthropology**

*Helen Cho, PhD\*, Davidson College, Davidson, NC 28035*

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**WITHDRAWN**



## **A110 Out With the “-Oids”: Problems With the Use of Racist Terminology in Forensic Science**

*Elizabeth A. DiGangi, PhD\**, Binghamton University, Binghamton, NY 13902; *Alba E. Craig, BA*, Indianapolis, IN 46227-1973; *Daniela Santamaria Vargas, BA*, Binghamton University, Binghamton, NY 13902

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**WITHDRAWN**



## **A111 Increasing Disparities: Sociopolitical Implications for the Use of Ancestry Estimation in the Medicolegal System**

*Jesse Roberto Goliath, PhD\**, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96853; *Meghan-Tomasita J. Cosgriff-Hernandez, PhD\**, La Mesa, CA 91941-4566

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**WITHDRAWN**

## A112 Don't Let Your World Be Too Small: The Relevance of Identity and Skeletal Populations

Phoebe R. Stubblefield, PhD\*, University of Florida, Gainesville, FL 32610

**Learning Overview:** After attending this presentation, attendees will be able to describe social or economic factors influencing racial self labeling in the United States. Attendees will be able to summarize sources of population data and forces affecting skeletal population formation and apply the data to a local circumstance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a decedent-focused approach to skeletal identification and illustrating a model for professional development of the concept of “forensic population served.”

If the goal of biological profile development is repatriation of the decedent, then knowledge of the population one serves is a requirement. This presentation describes the social and economic relevance of race labeling in the United States, as if race is a label that should be applied with the goal of intersecting with the decedent family’s use of the label. The ability to have a dynamic concept of race is improved by knowledge of historical and recent demographics and economies.

In this presentation, the concept of forensic catchment, or the forensic population served, is emphasized as a component of professional training that is a combination of: (1) historical and current population demographics; (2) local, national, and/or historic economic practices featuring human remains; (3) social forces affecting identity and local mortuary practices; and (4) the proximal circumstances of the death event. Catchment awareness must be trained or experienced. One of the consequences of higher education is employment in a location other than the one in which one was raised or trained. Without training, knowledge of the population served may be based on a narrow range of experience, years of experience, or exposure to a demographic shift. Considering appropriate social variables for each skeleton is the challenge, but the benefit is successful repatriation or a meaningful disposition.

Forensic anthropologists are good at identifying biological affiliation, and that affiliation may align with race labels in locations where race identification is not dynamic and where populations are not underrepresented in statistical databases.<sup>1</sup> Challenging circumstances include metropolitan areas with diverse populations and a generous supply of social labels, and individual circumstances where racial identity is strongly influenced by skin color. In the latter circumstance, racial classification will fail to repatriate the individual whose cranium is morphologically of African descent but whose skin is light enough to access a more beneficial social or economic status.

Focusing on repatriation of remains may require consideration of non-skeletal features such as associated clothing and artifacts, the location where the remains were found, the demographics of the neighbors, and the local economy. At minimum, applying this focus requires knowledge of the demographics in the reasonable geographic area. Reasonable is defined by context, including personal effects or artifacts found with or on the remains.

The multi-field anthropological approach empowers forensic anthropologists to communicate with death investigators in scope beyond skeletal variation, including interpretation of the relevance of property and demographic data. This knowledge may not confirm an identity, but it can assist the death investigator by providing directions for contacts and interviews. One of the benefits of anthropological training is that the anthropologist recognizes the internal versus the external point of view. When point of view is an aspect of race labeling of human remains, the consideration is “Will a label I apply agree sufficiently with the family’s knowledge of the decedent that the remains can be identified?” This may be a conceptual hurdle. From this perspective, the focus of biological profile analysis orients to the benefit of the decedent’s family, not to completing a scientific puzzle or exploring a skeleton.

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- <sup>1</sup> Ouseley S., Jantz R., and Freid D. (2009). Understanding Race and Human Variation: Why Forensic Anthropologists are good at identifying race.” *AJPA* 139:68-76.

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### Racial Identity, Decedent Identity, Skeletal Population



## **A113 Confronting Admixture: An Analysis of a Problematic Tradition in Forensic Anthropology**

*Teresa V. Wilson, PhD\**, Louisiana State University, Baton Rouge, LA 70803; *Sara Mire, BA*, Delcambre, LA 70528; *Bridget A. Cone*, Minden, LA 71055

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**WITHDRAWN**

## A114 Adulteration of Ancestry: A Time for Reform

*Ann H. Ross, PhD\**, North Carolina State University, Raleigh, NC 27695-7614; *Marin A. Pilloud, PhD\**, University of Nevada, Reno, Reno, NV 89557

**Learning Overview:** After attending this presentation, attendees will gain a better understanding of how ancestry has been used and misused over the past three decades.

**Impact on the Forensic Science Community:** This study will impact the forensic science community by offering suggestions on how to move forward with the practice of ancestry estimation and call for a reform of our current praxis.

In 1992, Norm Sauer called for a paradigm shift in forensic anthropology in which practitioners would move away from the socially loaded term “race” and replace it with “ancestry.”<sup>1</sup> This shift was meant to mark a move from social constructions of race toward recognition of geographically patterned human variation that could still be used within a forensic context. While many heeded the call and shifted toward ancestry in their research and reports, the actual approach to research and analysis did not change. This presentation provides an overview of the application of ancestry estimation and the debate surrounding race and ancestry over the past three decades as it applies to the work of forensic anthropologists.

The 1990s were witness to a resurgence of the study of race within biological anthropology. Lieberman and colleagues published survey results that showed that 50% of physical anthropologists accepted and 42% rejected the race concept, prompting Goodman and Armelagos to state that, “physical anthropology of the 1990s looks suspiciously like the physical anthropology of the 1920s and 1930s.”<sup>2,3</sup> Amid this renewed interest in research on race, there were also large-scale humanitarian crises in Rwanda and the former Yugoslavia that highlighted the need for population-specific standards within forensic anthropology for many parameters of the biological profile.

In response to the previous decade, between 2001 and 2010, there was a large growth in ancestry estimation method development. There was some renewed reflection on ancestry estimation as this terminology is largely embraced in forensic anthropology replacing the term “race.” Yet, even with this shift, the practice of ancestry estimation does not adequately encompass evolutionary theory in biological analyses. Further, the practice is broadly defended and accepted within the discipline with little critical reflection.

In the past ten years, there has been an increase in the validation of methods largely in response to the National Academy of Sciences 2009 Report.<sup>4</sup> In these publications that serve to validate ancestry estimation methods, there is little critique of the “race concept” or discussion of modern human variation or reference samples. Instead, the methods of ancestry estimation are tacitly accepted through the validation process. This decade has also seen an explosion of online (mostly unvalidated) programs in which ancestry can be estimated, further indicating the broad acceptance of the practice within our discipline and affirming its place in our practice.

Moving forward, forensic anthropologists need to reckon with the practice of ancestry estimation as it is currently envisioned. We are calling for another shift in the axiom that focuses on evolutionary theory, population history, and population-level reference samples. This practice can move to an analysis of population affinity and abandon the terms “ancestry” and “race” completely. Population affinity is a statistical estimate based on inherited phenotypic traits of the human skeleton that have been shaped by microevolutionary forces. This is not geographic ancestry, which all too often can be seen as interchangeable with social race and as a validation of the biological concept of race. It is time to acknowledge the social and scientific implications of the current practice of ancestry estimation and re-frame our approach to studying and analyzing modern human variation.

### Reference(s):

1. Sauer N.J. Forensic anthropology and the concept of race: If races don't exist, why are forensic anthropologists so good at identifying them? *Soc Sci Med.* 1992;34(2):107-11.
2. Lieberman L., Stevenson B.W., Reynolds L.T. Race and anthropology: A core concept without consensus. *Anthropol Educ Q.* 1989; 20(2):67-73.
3. Goodman A.H., Armelagos G.J. The resurrection of race: The concept of race in physical anthropology in the 1990s. In: Reynolds L, Lieberman L, editors. *Race and Other Misadventures: Essays in Honor of Ashley Montagu in His Ninetieth Year.* Dix Hills, New York: General Hall; 1996;174-86.
4. 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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### Race, Ancestry, Population Affinity



**A115 The Accuracy of Forensic Anthropological Ancestry Estimation in Modern Casework: The Evaluation of Forensic Anthropology Reports From Three Medical Examiners' Offices in the United States**

*Hillary R. Parsons, PhD\*, Odyssey Anthropology, Blaine, WA 98230*

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**WITHDRAWN**



## **A116 Race/Craft in Forensic Anthropology**

*Rachel Watkins, PhD\*, American University, Rockville, MD 20852*

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**WITHDRAWN**



## **B1 A Novel Colorimetric Isothermal Amplification Method for Cost-Effective, Rapid Body Fluid Identification**

*Tiffany R. Layne, MS\*, University of Virginia, Charlottesville, VA 22904; An-Chi Tsuei, MS, University of Virginia, Charlottesville, VA 22904; Annie Piland, BA, University of Virginia, Charlottesville, VA 22903; Nathan Tanner, PhD, New England Biolabs, Inc, Ipswich, ME 01938; James P. Landers, PhD, University of Virginia, Charlottesville, VA 22904*

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**Learning Overview:** After attending this presentation, attendees will have been introduced to a simple isothermal amplification method with colorimetric analysis, the use of novel messenger RNA (mRNA) targets for differentiation of body fluids, and how the integration of these two scientific advances applies directly to forensic body fluid identification. The 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 has value for adapting to other areas of forensic science and/or newer technology platforms.

Loop-Mediated Isothermal Amplification (LAMP) is ideal for qualitative assays because of its high specificity using two to three pairs of primers, generates multi-sized amplicon, and successful amplification can be read out colorimetrically. This study shows this technique can be directly applied to an important forensic application—body fluid Identification (bflD). Accurate presumptive and confirmatory tests are essential for gaining contextual information for crime scene investigators. False positive results are not uncommon with biochemical-based tests demonstrating a lack of specificity. In addition, many methods are known to be destructive to the sample and/or inhibit downstream procedures. Last, the analyst determines if the identification tests are positive or negative based on a color result, but this result is subjective due to differences that can occur from person to person.

This study demonstrates data on a LAMP method that is amenable to rapidly identifying body fluids. The panel includes venous blood, semen, and saliva and vaginal fluid detected with a colorimetric indicator and automated image analysis. The method is designed for easy implementation into the current forensic case workflow. Using mRNA targets for bflD, a high level of tissue- and human-specificity are derived from the use of multiple primer sets in the LAMP assay. The simplicity of this analytical method will minimally disrupt forensic labs performing DNA analysis, and the nature of the isothermal amplification massively reduces the complexity of instrumentation needed. Due to the use of phenol red in the reactions, an enclosed system was built with static lighting to analyze the reactions via camera or mobile phone. After an image is captured, it is analyzed for the hue value of each reaction, thus eliminating subjectivity from the analysis. This simple integrated system allows for minimal hands-on time with automated heating and image capture and analysis. Together the method and instrument show high promise as a forensic identification method.

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### **Isothermal Amplification, Body Fluid Identification, Colorimetric**



## **B2 Stay Gold: Lab-Based Considerations to Help Keep Forensic DNA Analysis the Shining Standard**

*Nancy Dinh, MS\*, Forensic Analytical Crime Lab, Hayward, CA 94545*

**Learning Overview:** The goal of this presentation is to discuss optimal approaches to biological evidence examination and sampling through the use of several illustrative case examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating ways in which a DNA analyst can expand evidence sampling techniques to produce test results that more accurately reflect the nature and amount of biological material present in the parent specimen.

DNA analysis has long been considered the gold standard of forensic science. Since the late 1980s, advances in Polymerase Chain Reaction (PCR) - based DNA technology have allowed analysts to derive more genetic information from progressively smaller amounts of biological material in less time. Today, nearly every step of the forensic DNA analysis process can be automated.

Some of the decisions most critical to successful DNA testing are made at the very beginning stages of the process: biological screening and sampling. For most of the myriad of physical evidence specimens one might encounter in a case, it is not feasible to automate these procedures. So, it is essential the procedures available to and the skills possessed by every forensic biologist are optimized for the best possible investigative outcome.

Several actual case examples will be presented to illustrate this approach. In the laboratory, a frequent request is to re-examine and re-test aged and/or minimal evidence specimens in numerous active and post-conviction cases, usually after another laboratory has been unsuccessful. This often requires the scientist to think “outside the box” and expand both the specimen type and sampling approach in order to glean sufficient biology to produce a meaningful DNA test result.

As an example, in a postconviction sexual assault case, a defendant was convicted solely based on eyewitness testimony. Examination of the victim’s underwear in 1987 revealed the presence of semen, but no DNA testing was performed at the time. Fifteen years later, the victim’s underwear was sent to another laboratory that concluded that no semen was present on the item. Nearly another 15 years later, the underwear was re-examined and, this time, semen was detected. The subsequent DNA testing result confirmed the convicted defendant as the semen source.

Each of the cases presented will reveal the value of specimen-specific examination and sampling techniques that not only produced dispositive results but also significantly higher amounts and quality of DNA from previously examined and sampled evidence. This evidence includes firearms and sexual assault specimens. In each case, this presentation will also explore the likely reasons why initial DNA testing was not successful.

These examination and sampling approaches are equally applicable to current, backlogged, cold, and post-conviction evidence testing. The results in these cases have benefitted both the prosecution and defense; some have reaffirmed the identity of a convicted suspect and others have resulted in the exoneration of innocents, but the overarching benefactor is justice. These cases will demonstrate that while forensic scientists must operate within a prescribed set of standard procedures, some of the most crucial decision-making in the DNA testing process must be autonomous.

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### **DNA, Evidence Examination, Sampling**

## **B3 An Aptamer-Based Fluorescence Biosensor for Salivary Lysozyme Using Localized Surface Plasmon Resonance-Enhanced Fluorescence of Zinc Selenide Sulfide (ZnSSe) -Alloyed Quantum Dots-Gold Nanoparticle Nanohybrid**

*Magda A. Pereira de Barros\**, University of Dundee, Dundee, Scotland DD1 4HN, UNITED KINGDOM

**Learning Overview:** The goal of this presentation is to provide a better insight on the use of nanomaterials in optical biosensor development for body fluid identification. More specifically, the advantages of the novel method over conventional detection technologies will be highlighted.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel analytical strategy based on the detection of saliva. By using lysozyme as a model analyte, this study has constructed an aptamer-based fluorescent nanobiosensor assay that is able to identify real saliva samples. This research has utilized the fluorescence emitting properties of Quantum Dots (QDs) as a signal reporter, the Localized Surface Plasmon Resonance (LSPR) fluorescence-mediating effects of gold nanoparticles (AuNPs), and the receptor binding effect of synthetic DNA aptamers to develop a highly sensitive and selective optical nanobiosensor for saliva. This presentation will open the door for better insight and further research on the use of nanotechnology in forensic science for body fluid identification.

**Introduction:** Saliva is a type of body fluid that can be collected as an evidence type within a crime scene context and can be used to identify an individual through DNA profiling. The identification of saliva is based on presumptive and confirmatory tests. Presumptive test such as Phadebas test<sup>®</sup> and SALIgAE<sup>®</sup> test are based on color change reaction that detects the enzymatic activity of amylase and is based on quick identification of the collected body fluid sample. Confirmatory tests, on the other hand (e.g., RSID<sup>™</sup> saliva), are based on immunochromatographic assays to confirm the identity of the saliva fluid detected presumptively. Despite the popularity of both methods, limitations such as poor sensitivity, poor selectivity, high cost, and destruction of DNA limits these methods. There is, therefore, an urgent need to develop new analytical methods to mitigate the limitations of existing detection technologies for saliva identification.

Fluorescent biosensors using nanomaterials that exhibit optical, magnetic, and/or electronic properties and that operate within a physico-chemical transducer interface with embedded bio-recognition elements hold the advantage of generating highly sensitive, highly selective, and rapid signals over conventional analytical tools for saliva identification.

**Summary of Results:** This study reports on the development of an aptamer-based fluorescence biosensor for saliva using ZnSSe-alloyed QDs and AuNPs hybrid nanostructure. Salivary lysozyme was used as a model enzyme analyte to optimize the biosensor assay for real human saliva sample detection. ZnSSe-alloyed QDs were synthesized and used as a fluorescent-emitting nanoreporter while citrate-capped AuNPs, a component of the hybrid assembly, was used to amplify the fluorescence signal via LSPR metal-enhanced fluorescence. Under optimum reaction conditions, salivary lysozyme was detected within 5min quantitatively and selectively, achieving a limit of detection of 28µg/mL. Application of the fluorescence-based nanobiosensor technology to detect real human saliva samples was successfully achieved.

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**Nanobiosensor, Saliva Identification, Lysozyme**

## B4 A Metabolomic Analysis for the Discovery of the Age of a Bloodstain Marker

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**Learning Overview:** After attending this presentation, attendees will understand that bloodstain metabolites can be used as a marker that makes it possible to estimate a specific time point of bloodstain age through this analysis that confirms changes in bloodstain metabolites over time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting a new perspective that not only hemoglobin, RNA, and protein (which have been the main targets of analysis in bloodstain age) but also metabolites can be used as a marker for the estimation of bloodstain age.

The purpose of this study is discovery of metabolite markers that can estimate the age of bloodstains in a week unit from the bloodstains aged up to 28 days. In blood collection, a total of 20 male and female donors, including both young and old, were recruited, considering the difference in blood metabolites according to sex and age. The obtained blood was dropped on filter paper to create bloodstains, and these bloodstains were kept in a dark room for 28 days while maintaining room temperature and humidity conditions.

Through High-Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) analysis, data on changes in bloodstain metabolites for the conditions of day 0, 7, 14, 21, and 28 were collected. A total of ten combinations were created by grouping two of the day conditions, each through volcano plot analysis and sparse Partial Least Squares Discriminant Analysis (sPLSDA), then comparative analysis between days was conducted. In the volcano plot analysis, Molecular Features (MFs) that passed fold change  $>2.0$  and p-value  $<0.05$  conditions were listed; then, in the sPLSDA, MFs constituting the first component that contributed the most to the distinction between the day conditions were listed. The average error rate of the sPLSDA was  $40 \pm 4.08\%$  (mean  $\pm$  standard deviation) in three combinations consisting of days after day 14 out of 10 combinations, but the error rate of the other seven combinations was 2.5%. As a result, a total of 57 metabolites showed statistically significant changes over time in the integrated MF list of the volcano plot analysis and the sPLSDA. Among these ten combinations, 31 metabolites were commonly found in six combinations. All these metabolites were not statistically significant on day 0 versus day 7, but significant changes were found on day 0 versus day 14, 21, 28, and on day 7 versus day 14, 21, and 28. Two metabolites appeared in common in seven combinations, showing the highest frequency. They not only showed significant changes in day 0 versus day 7, 14, 21, and 28 and on day 7 versus day 14, 21, and 28, but also confirmed that they were accompanied by a pattern of sustained increase or decrease over time.

Currently, metabolites are mainly associated with biometabolism and disease states. Therefore, these are analyzed in clinical studies. On the other hand, these are being studied as targets for drug analysis in forensic science. In this study, it was confirmed that metabolites in bloodstains can be used as a marker for estimating a specific time point of bloodstains aged within 28 days. Therefore, these markers will broaden the horizon of metabolite and blood component analysis in forensic science.

### Bloodstain, Age of Bloodstain, Metabolite

## B5 Evaluation Surface Effect of Bloodstains on Various Absorbent and Non-Absorbent Surfaces Using a Metabolomic Approach

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**Learning Overview:** After attending this presentation, attendees will understand that the recovery rate of components in the bloodstain varies depending on the surface of the bloodstain. Through this study, when collecting evidence in the field, it will be possible to refer to the priority of collecting bloodstain surfaces for a more accurate analysis of bloodstain components.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting a preliminary guide that provides a reference for the collection priority of bloodstain surfaces when analyzing bloodstain metabolites.

Bloodstains found at crime scenes contain immense information about the crime; thus, studies involving the analysis of small molecules in bloodstains have been conducted. However, most of these studies have not accounted for the difference in the results of small molecule analysis due to the surface of bloodstains. Therefore, it is necessary to observe the difference in components of bloodstains, which are extracted from different surfaces known as the “surface effect.” The purpose of this study is to identify the surface with the least effect and interference on the analysis of small molecules in bloodstains.

To evaluate the “surface effect,” this study prepared bloodstains on seven surfaces, including both absorbent (filter paper and mixed cotton) and non-absorbent surfaces (glass, tile, stainless steel, vinyl flooring, and wooden flooring). After collection of the bloodstain samples, metabolites were extracted from the bloodstains, then global small molecule analysis was performed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS). The multivariate analysis, such as heat map, correlation analysis, and Principal Component Analysis (PCA) was performed by Mass Profiler Professional (MPP) and MetaboAnalyst. Euclidean and Pearson distance metrics were used for heat map and correlation analysis, respectively. The common Molecular Features (MFs) were matched to the Agilent® METLIN PCDL B.08.00 database at the MS level using Agilent® MassHunter Qualitative Analysis.

To determine the ranking of the seven surfaces in the order of their similarity with blood, this study used three indicators: (1) count recovery rate (%) of MFs; (2) the number of MFs extracted from the surface without bloodstains; and (3) the difference in the abundance recovery rate (%) of MFs. The lower the sum of the rankings of all the results of each surface, the higher the ranking of that surface. Thus, based on a combined evaluation, this study found that the non-absorbent surfaces ranked better than the absorbent surfaces; wooden flooring was ranked as the most efficient surface, followed by stainless steel, vinyl flooring, glass, tile, filter paper, and mixed cotton. Additionally, this study confirmed the correlation between each surface and blood through multivariate analysis. Subsequently, non-absorbent surfaces tend to cluster more closely with the blood than the absorbent surfaces. This study will help in improving accuracy of small molecule analysis for bloodstain collection in the field in forensic science.

### Bloodstain, Surface Effect, Metabolomics

## **B6 The Development and Validation of a Multiple Reaction Monitoring (MRM) Mass Spectrometry (MS) Assay for Confident Identification of Protein Biomarkers for Blood, Semen, and Saliva**

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**Learning Overview:** After attending this presentation, attendees will understand the components of a protein MS assay for identification of body fluids as well as the necessary controls and data analysis requirements for use of targeted MS proteomics in a forensic context.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a complete assay for the identification of body fluids by protein markers for implementation in forensic casework.

As the amount of DNA needed for identifying individuals from forensic evidence becomes increasingly small, the need to know the source of that DNA (e.g., blood, saliva, or semen) becomes increasingly important—affecting both the defense and prosecution, as well as the public’s confidence in the accurate presentation of evidence and the impartial administration of justice. While this need is well recognized in the forensic community, currently there are no confirmatory body fluid assays in routine use in forensic laboratories. Proteins offer several advantages as body fluid biomarkers. The protein markers selected for this assay have tissue-specific functions (e.g., hemoglobin in blood to carry oxygen, amylase in saliva to digest carbohydrates, and semenogelin in semen for reproduction) and are therefore predominately and abundantly expressed in their respective body fluids, giving them both specificity and sensitivity as markers. Proteins are relatively stable to time and environmental insult, and because proteins markers do not require amplification, they can be detected even when partially degraded. MS-based protein assays are unbiased—the same protocol is applied to all unknown samples. Finally, protein and DNA can be separated from the same sample and, therefore, a protein body fluid assay does not consume DNA evidence that can be used for Short Tandem Repeat (STR) identity testing.

Previous work on proteomic body fluid identification established proof of concept and selection of specific protein biomarkers using an untargeted, discovery proteomics approach.<sup>1</sup> Discovery proteomics methods prioritize detection and identification of as many proteins as possible, with resulting limitations in sensitivity and reproducibility. In contrast, targeted proteomic techniques require the desired analytes to be defined in advance but are fast, sensitive, and reproducible, and thus more suited for standardization, validation, and routine implementation. Presented here is a detailed explanation of the steps taken to develop discovery study results into a targeted proteomics assay with standardized protocols and decision criteria suitable for thorough validation and implementation in routine forensic casework. This approach takes into account both the requirements of a sound targeted MS analysis method, supported by the large body of work in medical and pharmaceutical research, and the specific requirements for forensic testing of unknown samples and production of legally defensible results.<sup>2-5</sup>

The targeted assay uses MRM, carried out on a triple quad MS and involves targeting and isolating pre-selected peptide masses, fragmenting the selected peptides, and measuring the intensity of the expected fragment ions. Criteria for selection of peptides for each protein marker include stability, consistent ionization, avoidance of post-translational modifications, specificity to the target protein, and in the case of forensics, specificity to the target species. Fragment ion selection requires ruling out any masses that could be confused with interfering signals from the sample matrix, which in forensic samples may be unusually complex.

Using data collected from known samples of blood, semen, and saliva, as well as negative controls, a set of standard operating procedures was developed for the laboratory processing of forensic samples for analysis by High-Performance Liquid Chromatography (HPLC) and MRM MS. These include a quality monitoring system of known standards, controls, and expected instrument metrics. Similarly, a data analysis pipeline was developed, and statistical analysis of known samples was used to create robust decision criteria for the confident identification of at the level of targeted peptides, protein biomarkers, and, ultimately, the presence of a body fluid in an unknown sample.

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### **Proteomics, MRM, Body Fluid ID**

## **B7 Unlocking Dependable Forensic Results From Shell Casings: Advances in Method Development, Sample Collection, and Genetic Analysis**

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**Learning Overview:** After attending this presentation, attendees will have gained up-to-date knowledge regarding the use of genetic forensic techniques for spent shell casing samples in the United States. Attendees will also better understand emerging techniques for sample collection, extraction, and analysis. This includes an update on the general types of analysis currently performed as well as emerging methods. This presentation will demonstrate data resulting from several of these emerging methods, providing attendees with a realistic outlook on what is possible for this very challenging type of analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a foundational summary of shell casing analysis in United States crime labs. This presentation will further build upon this foundation by illustrating emerging techniques for collection, extraction, and analysis of genetic molecules on spent shell casing evidence. This presentation will provide statistically defensible data illustrating the use of these emerging techniques. The competence of attendees will be impacted by demonstrating the current state-of-the-art in shell casing analysis and by presenting new methods that may lead to the adoption of advanced shell casing extraction techniques in forensic labs that currently avoid this type of analysis due to poor past results. The performance of attendees will be impacted by demonstrating methods that could substantially improve shell casing extractions in laboratories that do accept and process this challenging type of evidence.

Extreme heat, reactive chemistry, and trace DNA deposits combine to make DNA analysis of shell casing evidence a tremendous challenge. While some laboratories process shell casing evidence, this practice is generally perceived as high risk, and many labs have opted to not routinely accept this sample type as a result. To overcome these challenges, a new set of capabilities based on the fundamental biology and chemistry associated with touch samples on fired brass casings has been assembled. This recent work demonstrates multiple significant advancements in the preparation, processing, and analysis of these samples that build upon other recent reports of shell casing evidence analysis success.

This study first developed a method for applying artificial fingerprint samples on brass shell casings to overcome issues associated with the high degree of variability associated with human touch samples. This enabled the precise quantitative evaluation of DNA yield and quality from simulated touch samples. The use of artificial fingerprint samples allows for more rapid and meaningful method development as different collection and purification methods can be directly compared in a quantitative fashion. This new approach reduced oxidative damage that previously rendered artificial touch samples too degraded for useful analysis. This approach routinely recovers 25%–30% of the DNA deposited in artificial fingerprints from shell casings with low degradation index values using conventional collection and extraction techniques.

Three collection approaches were compared using both artificial fingerprint and human touch samples on shell casings: (1) a standard flocked fiber swab; (2) Signature Science's casework-validated Forensic Recovery of Identity from Shell Casings (FRISC™) method that combines collection buffer submersion and swabbing; and (3) a novel submersion method engineered to simplify handling, block gunpowder residue contamination, decrease collection buffer volume, and enable parallel collection of protein for genetic protein variant analysis. Fired shell casing samples were processed by each of these methods, and DNA yields, degradation indexes, and Short Tandem Repeat (STR) detections were calculated for each replicate. All three methods proved to be robust, yielding DNA with slightly elevated degradation compared to non-brass surfaces, but of sufficient quality and quantity to generate at least partial STR profiles. For example, the FRISC™ method produced Combined DNA Index System (CODIS) -eligible profiles 38% of the time from fired shell casings ( $n = 21$ ) using Capillary Electrophoresis (CE).

This study additionally analyzed samples using Next-Generation Sequencing (NGS) to compare the utility of NGS and CE for brass samples. Generally, CE identified a greater number of CODIS markers and a greater percentage of the targeted markers in the kit. However, NGS often identified significantly more markers in total, especially when greater than 70pg of DNA was available for analysis. It is unclear whether these results highlight a sensitivity limitation for the NGS kit or arise from the NGS manufacturer-directed lower DNA input volume. No significant increase in allele calls was observed for shorter amplicons, reflecting modest overall DNA degradation. Taken together, these collection and sample preparation methods significantly advance the ability to obtain defensible forensic genetic results from fired shell casing samples.

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### **Shell Casings, DNA Analysis, Touch Sample**



## B8 Searching for the Silver Bullet of a Unified System to Deliver Forensic Science

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**Learning Overview:** After attending this presentation, attendees will have learned about practical, operational examples of how forensic science systems have moved toward a more holistic approach to deliver forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring how selected laboratories and agencies are undertaking changes in recognition of the importance of the crime scene component to the forensic science system. More integrated and holistic approaches are being introduced to improve the ability to identify the most probative samples and produce more meaningful results, with the aim of better use of resources and reduced risks of miscarriages of justice.

The silver bullet of a unified system to deliver forensic science has yet to be broadly implemented. Most existing initiatives focus on research to bring about improvements in laboratory testing. High standards are needed from forensic science, but this cannot be guaranteed from these initiatives alone. One cannot assume that every sample submitted to a forensic science laboratory has the same intrinsic value.

One of the functions of forensic science is to shed light on a past crime event. This requires acceptance of the importance of the scene. As test methods have developed, particularly in the biological area of DNA, the assumption is made that an association between a person and the scene is sufficient. Answering the question who, even when that is possible, is often insufficient to assist in solving the crime. Information on how and when is also needed. Association between scene sample and suspect is just as invalid as accepting correlation as causation. Knowledge of how and when materials transfer is vital and should be just as important as analytical protocols are for a forensic scientist.

If this truism is accepted, it is necessary to review how this knowledge is accumulated, disseminated, and used.

Systems have developed across the world in which crime scene examiners and laboratory personnel are separate groupings, sometimes with limited interaction. It is worth reflecting on how such systems have developed, given the importance of the scene. This presentation explores how certain laboratories and agencies are changing in recognition of this need for integration. Some agencies are introducing more holistic approaches in order to identify the most probative samples and produce more meaningful results.

Research is needed, and education is key in ensuring that all parts of the system from crime scene to court have a shared understanding of the capabilities and limitations of physical findings. All stakeholders need to go beyond validation of test and recovery methods to focus on the more difficult challenges in forensic science. At the moment, the tendency in every sector is to focus on aspects that lend themselves to McDonaldization and focuses on process over outcome. There is clearly a market for such an approach, but in doing so, it is vital to recognize that this is not appropriate for all crimes, and that using the output from a highly processed test facility runs the risk of using the answers to easy questions to incorrectly address the more difficult questions. Although there is no silver bullet of a unified system to deliver forensic science, exploring integration and holistic approaches to forensic science will help to find real ways to improve this complex system.

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### Laboratory Testing, Case Coordination, Crime Scene



## B9 Evaluating a Test for Shedding Propensity Using Tape Lifts From Different Skin Locations

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**Learning Overview:** After attending this presentation, attendees will have learned about tape lifting as a method for individual shedding propensity determination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing leads toward a standardized method to test shedding propensity that could be relevant in casework.

The shedding propensity of a person can assist data interpretation in casework when assessing the possibility of passive transfer for DNA analysis results. Szkuta et al. concluded that “the relative shedding ability” of the two contributors influenced the detection of DNA of both participants, and the DNA of a high shedder was detected both after active and passive transfer.<sup>1</sup> In some cases, a single-source DNA profile of a high shedder was generated from a surface after passive transfer.<sup>2</sup> This means that knowing the shedder status of a suspect will assist with case interpretation. Currently, there are different methods to look at shedding propensity, all based on evaluating palmar skin (finger) deposits, but there is no standardized method, especially for an uncooperative suspect. This project tested the feasibility of testing sebaceous skin for shedding propensity.

Eight different skin types and samples were collected from 15 males and 15 females over three non-consecutive days with D-squame adhesive tape disks; the samples were washed and unwashed fingers from both hands, toe, and sebaceous skin areas (arm, ear, and nape). Samples were extracted with the QIAmp® DNA Investigator kit and quantified with either Quantifiler® Trio or Human Plus (HP) kits. All samples were amplified using GlobalFiler® Polymerase Chain Reaction (PCR) amplification kits, typed on a 3500 genetic analyzer and evaluated for the presence of DNA mixtures. The quantitation values were then modified by subtracting the foreign DNA percentage. DNA concentrations for different skin sample types were log transformed and tested for Pearson r correlation values.

The neck area below the ears showed the highest average DNA concentrations over all three collections, followed by nape and unwashed fingers. DNA concentrations for washed fingers were always lower than for unwashed fingers for both hands. Arm and toe samples had the lowest average concentrations. The ear and arm samples showed the lowest variability across all three collections as measured through the relative standard deviation. Unwashed fingers had a lower relative standard deviation than the washed fingers. The toe sample set was the most inconsistent.

The DNA concentrations for different skin locations show different degrees of correlation. For palmar skin, washed and unwashed fingers show a strong correlation to each other. Comparing sebaceous to palmar skin, moderate correlations are shown in sample pairings of arm with right unwashed and washed fingers, and nape with right washed fingers. Only weak correlations were found for the sebaceous skin types to each other and for the toe samples.

Results confirms earlier data regarding a correlation between average DNA concentrations over all three collections for arm and finger samples. But looking at each individual collection, only one of three days showed moderate correlations. Donors did not easily fall into different shedding propensity categories. Individual DNA values showed a continuous distribution from low to high concentrations, except for the top two or three DNA concentrations. For left washed fingers these “high shedders” stayed the same over all three weeks, while right washed fingers showed some consistency, and the arm samples none at all. As of now, tape lifts from washed fingers seem to be a possible tool to determine shedding propensity and further work will be needed on sampling other areas.

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### Tape Lifts, DNA Shedding Propensity, Reproducibility

## **B10 Non-Destructive, Rapid Differentiation of Cell Types Relevant to Sexual Assault Investigations Utilizing Morphological and Autofluorescence Signatures**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of morphological and/or autofluorescence signatures of cells and their potential for identifying source tissue in sexual assault evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel method of differentiating cell types common to that of sexual assault samples. This can provide more probative information compared to traditional serological analyses and enhance the value of related DNA profiles.

Current methods for confirming the presence of spermatozoa in sexual assault samples can be time-consuming, lack sensitivity, and are not always conclusive; however, this remains the most definitive test for the occurrence of semen. Additionally, male DNA can be deposited without the presence of intact sperm, as may be the case with seminal fluid from vasectomized individuals or sexual activity where seminal fluid is not recovered (e.g., perpetrator wears a condom, digital penetration, penetration without ejaculation, etc.). The ability to detect bodily fluids, including semen, rapidly as well as quantify its presence in a sample could aid in forensic DNA analysis by limiting the amount of time spent performing serological testing as well as screening for probative samples for DNA profiling. Utilizing unique morphological and/or autofluorescence cellular signatures can be a rapid and non-destructive method for cell type differentiation; however, the application in forensic science casework has yet to be implemented. Thus, the goal of this study was to survey these novel signatures in four major cell types associated with sexual assault casework (vaginal, rectal, and penile epidermal cells, and seminal fluid) to develop a method to rapidly analyze these signatures in biological evidence.

For this study, the samples were collected from six males and six females and dried for at least two weeks prior to analysis. Cell populations were analyzed from single-source swabs of vaginal, rectal, and penile cells, as well as swabs of seminal fluid. The samples were extracted from the swabs and analyzed using Imaging Flow Cytometry (IFC) with five different excitation wavelengths and six detector channels ranging between 430nm–780nm, including a brightfield channel. Signatures for each cell type were constructed from ~60 different measurements capturing the size, morphology, and autofluorescence profiles of individual cells (e.g., area, aspect ratio, contrast, intensity, circularity). Finally, linear discriminant analysis was used to develop a quantitative framework for differentiating cell populations and predicting cell type within unknown/blinded samples.

Results showed that semen cell populations could be clearly differentiated from rectal, penile, and vaginal cell populations. Blinded samples of semen that were not included in the original analysis were analyzed and correctly identified as derived from a semen cell population with an accuracy of over 90%. To simulate cases where semen is not expected, this study also compared epithelial cell types (vaginal, rectal, and penile epidermal). Penile epidermal cells were clearly differentiated from rectal and vaginal cells with an accuracy greater than 90%. Interestingly, differentiation was also observed between semen cell populations derived from different donors, providing additional possibilities for how IFC may be applicable in forensic casework. These possibilities include donor differentiation in casework where there are multiple accused individuals or a consensual partner as well as potential for separating cells and developing individual donor DNA profiles from each cell population. The results obtained indicate that each cell type has unique and distinctive cellular signatures that can be detected in a rapid and non-destructive manner. Utilizing these features could aid forensic science casework in identifying the source of DNA in sexual assault evidence and also in predicting DNA yield and/or cellular separation with further research endeavors.

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### **Cell Differentiation, Serology, Sexual Assault Samples**

## B11 The Differentiation of Hemp From Marijuana Using a Qualitative Decision-Point Assay

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**Learning Overview:** After attending this presentation, attendees will understand how qualitative decision-point assays can be used to differentiate hemp from marijuana and will gain insight regarding the analytical challenges associated with the development, optimization, and validation of these assays.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the performance of a qualitative assay to differentiate hemp from marijuana.

*C. sativa* is the primary species of the *Cannabis* genus and can be broadly classified as hemp (fiber-type cannabis) and marijuana (drug-type cannabis). Regulatory changes following the passage of the 2018 Farm Bill define hemp as *C. sativa* containing no more than 0.3%  $\Delta$ -9-Tetrahydrocannabinol ( $\Delta$ -9-THC) on a dry weight basis. As a result, forensic laboratories must deploy methods to differentiate legal hemp from illegal marijuana. Due to the volume of submissions within the controlled substance discipline, this requirement presents a significant challenge for operational laboratories.

The United States Drug Enforcement Administration was the first to implement an analytical scheme that incorporates a decision-point approach to differentiate hemp from marijuana. Using an administratively set threshold, a qualitative assay can be used to identify  $\Delta$ -9-THC in plant material.

This presentation will describe the evaluation of multiple internal standards, extraction conditions, potential interferences, and instrumental parameters. Two analytical methods using Gas Chromatography/Mass Spectrometry (GC/MS) are described and their performance is compared. Optimized methods using an administrative threshold equivalent to 1%  $\Delta$ -9-THC were validated in terms of selectivity, limits of detection, repeatability, reproducibility, accuracy, dilution integrity, and carryover. *In situ* decarboxylation of  $\Delta$ -9-tetrahydrocannabinolic acid and cyclization of Cannabidiol (CBD) were also assessed.

$\Delta$ -9-THC was extracted from cannabis plant material using methanol.  $\Delta$ -9-THC-D3 was added post-extraction and samples were analyzed using dual Selected Ion Monitoring (SIM)/scan acquisition. Two analytical methods were developed to allow for the separation of common cannabinoids (~6mins) and a more expansive list (~12mins). Extraction efficiencies from plant matrix were 80%–92% and decarboxylation rates of 62%–67% were observed using an inlet temperature of 250°C. Using the 12-minute assay, the limit of detection in plant material was 0.3%  $\Delta$ -9-THC by weight, and linear detector responses were observed up to 10%  $\Delta$ -9-THC. Dilution integrity was established for plant materials containing up to 50%  $\Delta$ -9-THC. Selectivity was demonstrated using Cannabidiol (CBD), Cannabichromevarin (CBCV), Cannabicitran (CBT), Tetrahydrocannabivarin (THCV), Cannabivarin (CBV), Cannabicyclol (CBL), CBD, Cannabichromene (CBC), *exo*-THC,  $\Delta$ -8-Tetrahydrocannabinol ( $\Delta$ -8-THC),  $\Delta$ -6a,10a-Tetrahydrocannabinol ( $\Delta$ 6a,10a-THC),  $\Delta$ -9-THC,  $\Delta$ -10-Tetrahydrocannabinol ( $\Delta$ -10-THC), Cannabigerol (CBG), and Cannabinol (CBN). Repeatability and reproducibility at the decision-point yielded relative standard deviations of 3.4% ( $n=10$ ) and 5.1% ( $n=50$ ). Accuracy of the method was established using marijuana plant material provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (0.12% to 10.1%  $\Delta$ -9-THC) and five commercial hemp samples. Replicate analyses were performed using the 15 sources of plants. Sensitivity, specificity, negative predictive value, and positive predictive value were 100% ( $n=70$ ). Extracts were stable for five days when refrigerated in the dark. Potential interferences from CBD were evaluated using plant matrix fortified to the equivalent of 50% CBD by weight. Negative controls, positive controls, and plant extracts were free from interferences. Finally, measurement uncertainty was estimated at 0.3%  $\Delta$ -9-THC (below the administrative threshold). The expanded uncertainty using a 95.3% confidence interval ( $k=2$ ) was 12.2% ( $0.3 \pm 0.04\%$   $\Delta$ -9-THC by weight of plant material).

While qualitative decision-point assays can be used to differentiate hemp from marijuana, overall assay performance can be impacted by decarboxylation rates, potential interferences, and reproducibility between instruments. Careful optimization and rigorous validation is required. Using this approach, the qualitative assay using an administrative threshold of 1%  $\Delta$ -9-THC was found to be robust and reliable.

### Hemp, Marijuana, GC/MS

## B12 The Results of an Interlaboratory Validation to Differentiate Marijuana From Hemp

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**Learning Overview:** After attending this presentation, attendees will gain insight regarding the benefits of interlaboratory validations, the potential for differences in analytical performance between laboratories, and the need for full, independent, and rigorous validation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging interagency collaborations and interlaboratory comparisons as part of new assay development.

Following the passage of relatively recent federal and state legislation, laboratories must now differentiate marijuana from hemp based upon the concentration of  $\Delta$ -9-tetrahydrocannabinol ( $\Delta$ -9-THC) in the plant. The federal 2018 Farm Bill defines hemp as *Cannabis sativa* L. containing not more than 0.3%  $\Delta$ -9-THC on a dry weight basis. In order to avoid full quantitative analyses on routine plant exhibits, some organizations have adopted decision-point assays to differentiate illegal marijuana from legal or commercial hemp products.

In 2019, with support from the Texas Forensic Science Commission, Sam Houston State University, Houston Forensic Science Center, Harris County Institute of Forensic Sciences, and the Texas Department of Public Safety Crime Laboratory Service initiated a collaborative study to address the issue. Using a modification of the method first reported by the United States Drug Enforcement Administration (DEA), a qualitative decision-point assay was developed and validated. Using a 1%  $\Delta$ -9-THC administrative threshold, Gas Chromatography/Mass Spectrometry (GC/MS) was used to differentiate hemp from marijuana using a qualitative assay. Deuterated internal standard and dual Selected Ion Monitoring (SIM)/scan acquisition was used to analyze methanolic extracts of suspected cannabis plant material.

The method was fully validated at all four sites in terms of limit of detection, selectivity, precision, accuracy, dilution integrity, carryover, and extract stability. Decarboxylation of  $\Delta$ -9-tetrahydrocannabinolic acid to  $\Delta$ -9-THC was evaluated during analysis, and potential interference from Cannabidiol (CBD) was also investigated.

Selectivity was demonstrated using more than 12 cannabinoids, including those that are known to elute in close proximity to  $\Delta$ -9-THC. Selectivity and retention time stability was established over ten injections on one day and one injection over ten days at each site. Inter-assay Coefficient of Variations (CVs) ( $n=10$ ) ranged from 0.0%–3–0.31% for  $\Delta$ -9-THC and 0%–0.30% for all other cannabinoids. Limits of detection in methanolic extracts ranged from 0.008–0.015mg/mL (equivalent to 0.15%–0.3%  $\Delta$ -9-THC by weight of plant material). Although the assay is not quantitative in nature, linearity of detector response was evaluated at each site, yielding  $R^2$  values of 0.992–1.000. Repeatability and reproducibility of the 1% decision-point control were evaluated using the relative peak area ( $\Delta$ -9-THC/ $\Delta$ -9-THC-D3), yielding CVs of 1.9%–3.7% ( $n=10$ ) and 2.4%–5.1% ( $n=50$ ). Carryover of  $\Delta$ -9-THC was observed at the equivalent of 50% or more  $\Delta$ -9-THC at some sites. Secondary decision-point controls were utilized as a quality control measure. Dilution integrity was established using five- and ten-fold dilutions of extracts prepared from placebo matrix fortified with 20%–50%  $\Delta$ -9-THC.

Each of the collaborating laboratories selected an administrative threshold in plant material of 1%  $\Delta$ -9-THC by weight, rather than the 0.3% statutory threshold. In addition to this safeguard, the analytical approach is likely to underestimate the total  $\Delta$ -9-THC due to incomplete extraction efficiencies and decarboxylation rates. It should be noted that this conservative approach is designed to prevent false positive results and increase the specificity of the assay at the expense of sensitivity. Using plant materials of known  $\Delta$ -9-THC content, specificity and positive predictive value were both 100%. Of the 280 analyses, a total of eight false negative results were observed, lowering the overall sensitivity and negative predictive value to 94% and 95%, respectively. Extracts were found to be stable when stored refrigerated for up to five days. Potential interferences from CBD were evaluated using negative controls and plant extracts fortified with 0, 10, 20, 30, 40, and 50% CBD. No false positives were observed. Although the assay is qualitative in nature, measurement uncertainty was estimated at 0.3%  $\Delta$ -9-THC (below the administrative threshold). Expanded uncertainties using a 95.45% confidence interval ( $k=2$ ) were 12.2%–21.8% among laboratories, equivalent to  $0.3 \pm 0.04\%$  to  $0.07\%$   $\Delta$ -9-THC by weight.

Although satisfactory performance was achieved at all sites, differences in analytical performance were also observed for some variables. Therefore, although multi-agency collaborations and interlaboratory validations are extremely beneficial, they do not supplant the need for there to be a full, independent, and rigorous validation.

### Marijuana, Hemp, GC/MS

## **B13 An Analysis of Cannabis Plant Materials in Drug Enforcement Administration (DEA) Laboratories: A Year in Review**

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**Learning Overview:** After attending this presentation, attendees will better understand the application, benefits, and limitations of implementing a new analysis protocol for cannabis plant material.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a summary of results and trends observed throughout DEA laboratories during the year following implementation of a new analytical scheme for analysis of cannabis submissions.

Prior to December 2018, DEA laboratories used a three-tier analytical scheme for analysis of cannabis submissions that consisted of macro/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 analysis protocol provided the scientific foundation for reporting *Cannabis* conclusions. On December 20, 2018, with the signing of the Agriculture Improvement Act of 2018 (2018 Farm Bill), significant legal changes were implemented, including the definition of “hemp” 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.” The new law also excluded the term “hemp” from the definition of marijuana in the Controlled Substances Act. The change directly affected the manner in which suspected cannabis submissions seized after the Farm Bill were analyzed by the DEA laboratories and prompted the development and validation of a new analysis protocol.

The main objective of this presentation is to provide a summary of analysis results and trends observed during the year following implementation of the new cannabis analytical scheme. This assessment covers laboratory submissions seized after December 20, 2018, and analyzed between June 2018 and August 2020. During this period, over 62,000 single and multi-unit submissions were analyzed by DEA analysts, and approximately 9,000 of them were suspected cannabis submissions. After analysis using the new analytical scheme, 1,278 submission were reported as *Marijuana*, indicating that the morphological characteristics of the materials were consistent with cannabis, the THC:CBD ratio was above 3.0, and the total THC level in each individual unit analyzed was above the administrative decision limit of 1% (w/w). A total of 193 submissions were reported as *Inconclusive*, meaning that at least one unit within the exhibit did not fulfill the minimum criteria needed to report *Marijuana*. Inconclusive reports are generated when at least one of the three tests required by the new analytical scheme, micro/macrosopical analysis, 4-AP color test, and GC/MS analysis fails to produce a positive result.

This presentation will discuss the trends observed when inconclusive results are reported. Among others, the following questions will be addressed: What are the most common factors producing inconclusive findings? Are there regional/geographical differences influencing the results? Are distinguishing cannabinoid profiles observed and correlated with THC-rich and CBD-rich samples? Are low levels of THC always observed in CBD-rich samples? Are low levels of CBD always observed in THC-rich samples? What is the distribution of THC levels observed for hemp-like submissions? Furthermore, this presentation will also include a summary of the results obtained when a selected number of inconclusive samples are further analyzed to determine the total THC level using a Liquid Chromatography/Ultraviolet/Visible (LC/UV/Vis) quantitative method.

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### **Cannabis, Marijuana, Hemp**

## B14 Candies and Cannabinoids—The Rapid Identification of Cannabinoids in Plant Material and Edible Complex Matrices by Ambient Mass Spectrometry

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**Learning Overview:** After attending this presentation, attendees will better understand how ambient ionization mass spectrometric techniques can assist with the identification and differentiation of cannabinoids in complex plant and edible 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 identify plant material and food products that contain cannabinoids, while circumventing problems often encountered using conventional methods.

As cited in the National Institute of Justice (NIJ) 2019 *Report to Congress: Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices*, some of the greatest challenges to emerge in recent years are consequences of the “legalization and decriminalization of marijuana.”<sup>1</sup> Furthermore, the report indicates that the issues require the “implementation of new testing strategies,” and that “testing methods must be developed to test THC (tetrahydrocannabinol),” in a variety of plant-based substances, edibles, and extracts. Current protocols utilized by law enforcement and crime laboratories, among which there is little uniformity, can be cumbersome and often require extensive sample preparation, time-consuming samples runs, and complex data processing. This study focuses on the unique capabilities of Direct Analysis In Real Time-High-Resolution Mass Spectrometry (DART®-HRMS) for the rapid detection of cannabinoids in *Cannabis sativa* plant material and cannabinoid-infused complex edible matrices. This ambient, high-resolution technique permits direct analysis of samples in their native forms, as well as rapid interrogation of samples following their chemical alteration through exposure to derivatizing agents.

A wide variety of samples were investigated, including commercial Cannabidiol (CBD) products, edible Certified Reference Materials (CRMs) infused with cannabinoids, hemp plant material (which is a variety of *C. sativa*), and edible samples prepared in-house, such as candies, baked goods, and other food products. DART®-HRMS analysis rapidly reveals the presence of cannabinoids in the plant and edible products without sample preparation. While several cannabinoid molecules were detected, including Cannabigerol (CBG) and Cannabinol (CBN), each DART®-HRMS-derived spectrum exhibited a peak at  $m/z$  315.232, which is consistent with protonated THC and CBD. Because these two molecules share the same molecular formula of  $C_{21}H_{30}O_2$  and therefore the same protonated mass, they are indistinguishable by DART®-HRMS under soft ionization conditions without sample pretreatment. Furthermore, they remain indistinguishable even under Collision-Induced Dissociation (CID) conditions that induce fragmentation of the molecules. As such, an alternative approach must be taken to differentiate and identify these two cannabinoids. It was found that THC and CBD are readily distinguishable under both soft ionization and CID conditions when the derivatizing agent *N*-Methyl-*N*-(Trimethylsilyl)Trifluoroacetamide (MSTFA) was used. Engagement by the derivatizing agent of the single –OH group in THC and the two –OH groups in CBD converts the two compounds to molecules with protonated  $[M+H]^+$  masses of 387.272 and 459.312, respectively. This results in THC and CBD chemical standards producing distinguishable mass spectra through DART®-HRMS analysis. Furthermore, chemical signatures with additional cannabinoid-related information are provided when plant materials, commercial products, and edibles are analyzed by DART®-HRMS after derivatization. In summary, the data demonstrate that DART®-HRMS can detect and differentiate cannabinoids in complex matrices and provide a method to triage edible marijuana evidence in crime laboratories while circumventing problems often encountered using conventional methods.

### Reference(s):

1. National Institute of Justice (NIJ) 2019 *Report to Congress: Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices*. <https://www.justice.gov/olp/page/file/1228306/download>.

### Mass Spectrometry, Cannabinoids, Edibles



## B15 Raman Spectroscopy and Multivariate Curve Resolution for Mixture Analysis of Forensic Drug Samples

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**Learning Overview:** The goal of this presentation is to describe the potential use of Raman spectroscopy coupled with Multivariate Curve Resolution (MCR) for analyzing multi-component samples commonly encountered in seized drug exhibits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of Raman spectroscopy for the analysis of complex mixtures that are difficult to effectively analyze by bulk spectroscopic analysis.

In recent years, there has been a rapid proliferation of new compounds emerging in the sphere of recreational drug use. These compounds are typically developed in an effort to stay ahead of drug scheduling laws banning their use and distribution. Keeping pace with the identification of these compounds is an increasing challenge for forensic labs. Most of these compounds are synthetically produced and are typically structurally similar to existing psychoactive substances. The compounds are constantly evolving and the lifecycles in the illicit drug market can vary. These factors make forensic drug analysis a moving target that points to the need for higher-throughput analytical methods that are also cost effective. The wave of Novel Psychoactive Substances (NPS) has resulted in a multitude of structurally similar compounds and closely related isomers that can be difficult to distinguish by some techniques. Differentiating these substances based on their vibrational spectra is generally straightforward for relatively pure materials. With complex mixtures or sufficient dilution, however, the utility of these techniques can be greatly diminished.

The objective of this work was to investigate the use of Raman spectroscopy for the identification of multiple constituents in mixtures. The method is intended to be a non-targeted screening approach with limited sample preparation requirements. By exploiting compositional variations in powder mixtures at the micro-scale, estimates of pure spectra of the constituents can be resolved using MCR techniques. The estimated pure spectra can be utilized more effectively in library searches, and detection limits can be significantly improved compared to mixture spectra from bulk spectroscopic analysis.

One motivation for this work was the detection of fentanyl and associated analogs in the presence of common cutting agents; however, the technique would be applicable to a broad range of drug classes. In evaluating this approach, this study utilized a surrogate for fentanyl, N-Phenethyl-4-Piperidinone (NPP), as a target compound. NPP is an intermediate in the synthesis of fentanyl that shares structural and spectral similarities. Raman data was collected using 830nm excitation on binary blends of NPP with 13 common cutting agents associated with fentanyl and heroin. In addition, NPP blends with multiple cutting agents as well as several over-the-counter medications that were also investigated. NPP levels down to 5% in blends were investigated in these studies and was readily identified in all samples using MCR. The results of these studies will be presented. This technique is currently being extended to seized drug materials to determine the effectiveness in real samples. Results of these investigations will also be presented, if available.

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### Raman Spectroscopy, Drug Analysis, Chemometrics



## **B16 Benzodiazepine Drug Chemistry Analysis Results Submitted to the National Forensic Laboratory Information System (NFLIS-Drug): 2015 to 2018**

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**Learning Overview:** After attending this presentation, attendees will understand the findings from the Drug Enforcement Agency's (DEA) benzodiazepine drug chemistry results submitted to the NFLIS-Drug from 2015 to 2018.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic laboratories, toxicology laboratories, and medical examiner and coroner offices information regarding trends in benzodiazepines and emerging benzodiazepines submitted to NFLIS-Drug.

The NFLIS is a program of the Drug Enforcement Administration, Diversion Control Division. NFLIS-Drug systematically collects drug identification results and associated information from drug cases submitted to and analyzed by federal, state, and local forensic laboratories. These laboratories analyze controlled and non-controlled substances secured in law enforcement operations across the country. The NFLIS-Drug data are used to support drug scheduling decisions and to inform drug policy and drug enforcement initiatives nationally and in local communities around the country.

Results of national estimates of selected benzodiazepine drug cases that were submitted to state and local laboratories from January 1, 2015, through December 31, 2018, and were analyzed by March 31, 2019, will be presented. This presentation will also highlight selected benzodiazepines of interest, reports of selected benzodiazepines by state, regional trends, and counts of alprazolam and etizolam reported with other drugs in the same item (e.g., not necessarily true drug combinations).

Alprazolam and clonazepam are the top prescribed benzodiazepines in the United States. NFLIS-Drug also shows this trend because these two are the most common benzodiazepines reported by participating laboratories. From 2015 to 2018, an estimated 263,538 benzodiazepine reports were identified by state and local laboratories. Estimated benzodiazepine reports decreased by 14% between 2015 and 2018. Alprazolam and clonazepam accounted for 87% of benzodiazepine reports between 2015 and 2018.

Benzodiazepines are prescribed with narcotic analgesics and are co-involved in overdose deaths. Of the narcotic analgesics reported in the same item as alprazolam (16%), fentanyl (43 reports), fentanyl-related compounds (42 reports), and oxycodone (31 reports) accounted for the majority of substances. Some benzodiazepines have emerged that are not prescribed for therapeutic use in the United States and can be seen in NFLIS-Drug data. Some of these substances include phenazepam, etizolam, clonazepam, flubromazolam, and flualprazolam. Etizolam has been one of the most prominent emerging benzodiazepines over the years; it increased 99% between 2018 and 2019. An emerging benzodiazepine, flualprazolam, increased 1,565% between 2018 and 2019. Some of these emerging benzodiazepines have been reported in toxicology by the United Nations Office on Drugs and Crime in its *Current NPS Threats* report.<sup>1</sup>

Attendees will gain an understanding of the trends in benzodiazepines across the United States and how those trends complement other data sources. They will also recognize NFLIS-Drug as a comprehensive information system that includes data from laboratories that handle the nation's drug analysis cases. NFLIS publicly shares aggregated and analyzed data through various publications throughout the year, including national and regional trends of drugs submitted to state and local laboratories, as discussed in this presentation. These publications can benefit laboratories, law enforcement, public health agencies, and other stakeholders by increasing their awareness of emerging substances and other drug trends.

### **Reference(s):**

<sup>1</sup> United Nations Office on Drugs and Crime. *Current NPS Threats*. <https://www.unodc.org/unodc/en/scientists/current-nps-threats.html>.

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### **Benzodiazepines, Drug Trends, NFLIS**



## B17 Analyzing Designer Fentanyl Analogs Using a Fentanyl Classifier

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**Learning Overview:** After attending this presentation, attendees will have learned how mass spectral similarity mapping, as implemented in the Fentanyl Classifier, can be used to generate putative identifications of fentanyl analogs not contained in a reference library.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that drug screening with the Fentanyl Classifier has the potential to greatly reduce false negative presumptive identifications of fentanyl analogs that differ from fentanyl by up to two modifications by leveraging the increased information content available through mass spectral similarity mapping.

Fentanyl is a highly addictive synthetic narcotic originally developed for treating severe pain. Growth in abuse of fentanyl, fueled in part by designer fentanyl analogs, has led to an unprecedented rise in overdose deaths. The large and rapidly evolving number of these drugs presents a challenge to chemical identification. Mass spectral similarity mapping is a natural extension of traditional mass spectral similarity searching. In both processes, a query mass spectrum of an analyte is searched against a library of reference spectra. Whereas a traditional similarity search provides an analyst with a hit list—a high-confidence way of identifying an analyte with a replicate spectrum in the library—similarity mapping provides a map that is informative even for analytes that are not in the reference library, such as new designer drugs.

A library of mass spectra that included fentanyl and Type I fentanyl analogs was collected. Here, *Type* indicates the number of modification sites by which an analog differs from the standard fentanyl scaffold. Mass spectral similarity maps can be generated using query spectra and the fentanyl library. These maps can be scrutinized to classify the query based on the likely site of modification and, in some instances, propose a complete structure for the query. An open-source implementation of mass spectral similarity mapping applied to fentanyl analogs, referred to as the National Institute of Standards and Technology (NIST) Fentanyl Classifier (NFC), is available at <https://github.com/asm3-nist/FentanylClassifier>. The NFC was tested using several example spectra, including replicate spectra of fentanyl, replicate spectra of the Type I fentanyl analogs contained in the library, spectra of Type I fentanyl analogs not represented in the library, spectra of Type II analogs, and spectra of non-fentanyl compounds. As expected, the NFC correctly localized modification sites for fentanyl analogs that differ from fentanyl by modification on, at most, two sites (i.e., Type I and Type II fentanyl analogs). In cases where the query was a Type II fentanyl analog with *composing cognates* contained in the library, the NFC was able to accurately identify the query short of positional isomers.

### Fentanyl, GC/MS, Screening



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## **B18 National Institute of Standards and Technology (NIST) Direct Analysis in Real Time Mass Spectrometry (DART®-MS) Tools for Seized Drug Analysis**

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**Learning Overview:** After attending this presentation, attendees will have an understanding of how DART®-MS can be employed for screening seized drug evidence using updated NIST databases and software tools.

**Impact on the Forensic Science Community:** By leveraging the increased information content available through multiple in-source collisionally induced dissociation fragmentation spectra, drug screening using DART®-MS analysis has the potential to greatly reduce false positive presumptive identifications. This can prevent unnecessary subsequent analysis or better inform subsequent analysis, impacting resource and time management for seized drug evidence casework.

Over the past decade, forensic drug chemists have been faced with increasing case backlogs. This is in part due to the influx of synthetic opioids and other novel psychoactive substances, the complexity of which often lead to increased time required to confidently detect and identify these compounds. In an effort to speed up analyses, some laboratories have begun to implement Direct Analysis in Real Time Mass Spectrometry (DART®-MS). This technology is capable of detecting a number of forensically relevant compounds including drugs of abuse, explosives, chemical warfare agents, lotions and lubricants, and Toxic Industrial Chemicals (TICs) in seconds. While the technology shows promise, especially for forensic analysis of seized drugs, the data analysis necessary for confident presumptive identifications is currently cumbersome and impractical and often only incorporates a single, low-fragmentation spectrum.

NIST scientists have developed new tools to support the analysis of DART®-MS data, in particular, an updated NIST DART®-MS Forensics Database containing reference mass spectra measured at multiple orifice energies for several hundred compounds of interest, a software script for building custom DART®-MS databases with automated quality control measures, and an interactive software program designed specifically for interacting and searching DART®-MS databases. The updated reference DART®-MS database is freely available and is expected to be continually updated as mass spectra of drugs and other compounds of interests are acquired. The software tools are open-source and freely available. By using some or all of these tools, drug chemists can greatly improve their ability to provide presumptive identifications of seized drugs using DART®-MS, with subsequent impact on downstream confirmatory analyses.

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### **DART®-MS, Screening, Seized Drugs**

## B19 A Characterization of the Vapor Profiles of Fentanyl and Synthetic Opioids for Instrumental and Canine Detection

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**Learning Overview:** After attending this presentation, attendees will better understand the methods used for headspace analysis as well as similarities and differences in the vapor signatures of differing fentanyl and fentanyl-related samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the research to determine the vapor signatures of fentanyl and related analogs.

Fentanyl is a Schedule II synthetic opioid, approved for use as a painkiller and anesthetic, and known for its strong analgesic and euphoric properties. In recent years, there has been an escalation in fentanyl abuse as indicated by a 65% increase in fentanyl reports submitted to the National Forensic Laboratory Information System (NFLIS) from 2016 to 2017, making fentanyl the fifth most-frequently identified drug at crime labs by NFLIS in 2017, and drug seizures have continued to increase through 2019.<sup>1,2</sup> The ability to detect bulk product as it crosses our borders and prior to its street distribution is an important part of defeating the problem. However, the high potency of fentanyl is of great danger to users as well as law enforcement officers. Since ingestion of milligram amounts can cause an overdose, many enforcement agencies instruct their officers to avoid any direct contact with material suspected of containing fentanyl.<sup>3</sup>

Given this current environment, providing a safe and effective method for field detection of fentanyl and related substances for law enforcement officers and first responders is imperative. To minimize the risk, non-contact detection methods such as vapor detection are preferred. Vapor detection has the benefit of being non-contact and non-intrusive, although vapor sampling is only feasible when the target vapor is present at concentrations high enough to be detected. Though the low volatility of target analytes such as pharmaceuticals can limit instrumental vapor detection, this can be overcome through preconcentration sampling, such as using Solid Phase Microextraction (SPME). Biological detectors such as canines are other examples of highly efficient field vapor detectors and are frequently used in the detection of low volatility analytes such as explosives and narcotics. Canine detectors overcome low vapor availability by detecting the odors associated with the parent molecule instead of the parent molecule itself. The collection of Volatile Organic Compounds (VOCs) associated with the parent material can be referred to as its vapor signature.

SPME was used to extract volatiles from the headspace of solid fentanyl samples with analysis by Gas Chromatography/Mass Spectrometry (GC/MS). Following method optimization, the headspaces of fentanyl samples, to include pharmaceutical-grade as well as street exhibits, were measured. Analysis also included a lot-to-lot comparison of the pharmaceutical-grade material, in addition to evaluation to fentanyl analogs. Finally, forced degradation experiments, including thermal, oxidative, and acid degradation, were carried out to determine the origin of the analytes making up fentanyl's vapor signature.

A number of analytes were identified in the headspace of solid fentanyl. Analytes making up the vapor signature of fentanyl included benzaldehyde, pyridine, aniline, N-phenylpropanamide, and N-Phenyl-4-Piperidinone (NPP). In future research, the identified vaporous analytes will be used as targets for detection.

### Reference(s):

1. Drug Enforcement Administration, DEA-DCT-DIR-007-20 (Dec. 2019).
2. U.S. Customs and Border Patrol. *CBP Enforcement Statistics Fiscal Year 2020*. Retrieved from <https://www.cbp.gov/newsroom/stats/cbp-enforcement-statistics> on 07 July 2020.
3. Drug Enforcement Administration, DEA-DCT-DIR-040-17 (Oct. 2017).

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### Fentanyl, Opioid Detection, Headspace Analysis

## B20 High Concentrations of Illicit Stimulants and Cutting Agents Cause False Positives on Fentanyl Test Strips

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**Learning Overview:** The goal of this presentation is to educate fentanyl test strip users and harm reduction workers of the possibility of false positives when illicit stimulants and cutting agents are present in high concentrations. Attendees will become familiar with possible interference concentrations and procedures on proper sample dilutions to avoid false positives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing quantitative evidence of known illicit stimulants and common cutting agents that could be present in opioid and/or fentanyl samples and how their presence in samples could lead to false positives when using immunoassay Fentanyl Test Strips (FTS).

The evolution of the opioid epidemic has caused a series of opioid-related overdose deaths that can mainly be attributed to fentanyl combined with various illicit substances. Although the use of FTS has increased since the beginning of the epidemic, its role as a harm reduction technique has not been well established. Typical street samples are prepared for analysis using FTS by either filling a baggie or cooker with water to dissolve the sample prior to dipping a strip in the solution. Previous research fails to include common stimulants and cutting agents that could lead to the possibility of false positives in FTS. Due to the varying concentrations of cutting agents in street drugs, if the dilution of the raw sample is not enough, it could result in a too-concentrated sample that could cause a false positive on the test strip.

BTNX Inc.'s 20ng/mL immunoassay Rapid Response Fentanyl Test Strips were obtained and tested against four different pharmaceuticals (diphenhydramine, alprazolam, gabapentin, and naloxone buprenorphine) and three illicit stimulants (cocaine HCl, methamphetamine, and 3,4-Methylenedioxymethamphetamine [MDMA]) in concentrations from 20mg/mL to 0.2mg/mL. The FTS testing pad is divided into two sections: the control area and the test area where two bars indicate a sample negative for fentanyl and a single bar indicates a positive for fentanyl sample.

By eye, FTS with cocaine, alprazolam, gabapentin, and naloxone buprenorphine were negative even at the highest concentrations. FTS testing bands with methamphetamine, MDMA, and both diphenhydramine capsules and tablets did not appear when the analyte was in high concentrations (>2mg/mL). These samples could be read as false positives. The control and testing bands on the FTS were then quantitatively measured and compared to a threshold set by a fentanyl standard. The integrated intensities of test bands from solutions of cocaine, Deionized (DI) water, and tap water (which should all be negative for fentanyl) appeared well above the fentanyl threshold. The integrated intensities of the test bands from moderately concentrated samples of methamphetamine, MDMA, diphenhydramine capsules, and diphenhydramine tablets were below the fentanyl threshold, consistent with the visual false positive results. At or above these concentrations, the FTS is likely to produce a false positive result.

In conclusion, fentanyl test strips are commercially available and successful at detecting fentanyl to the specified limit of detection and can be a valuable tool for harm reduction efforts. Users should be aware that when potential drug adulterants are in high concentrations, the FTS can give a false positive result.

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### Fentanyl Test Strip, Stimulant, Cutting Agent

## B21 An Analysis of Drug Residue From Needle-Exchange Syringes in Washington, DC

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**Learning Overview:** The goals of this presentation are to: (1) impart knowledge of real-time Intravenous (IV) drug use in Washington, DC; (2) illustrate geospatial drug trends from used syringes in Washington, DC; and (3) compare geospatial drug trends from used syringes, death investigations syringes, and seized drug evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how a long-term implementation of this syringe surveillance program can offer a robust monitoring system of drug trends in Washington, DC, by combining with multiple laboratory data streams providing intelligence on IV drug-user practices and emerging dangerous substances.

This presentation will provide insight into geospatial drug trends in Washington, DC, compiled from the analysis of drug residue within used syringes, donated by needle-exchange programs. This data is compiled alongside existing data from death investigation syringes and seized suspected Controlled Dangerous Substance (CDS) exhibits. This study represents the first time an analysis of residual contents from used syringes collected from harm reduction services (needle-exchange programs) has been conducted in the District of Columbia. As drug abuse remains a complex, dynamic phenomenon, it is hypothesized herein that the triangulation of data from three different sources will allow for more comprehensive opioid monitoring.

Since the opioid epidemic was declared a public health emergency in 2017, increased knowledge of up-to-date opioid abuse, opioid trends, and novel emerging substances has been needed. What began as an outbreak of prescription drug abuse evolved into a second wave of heroin overdoses, followed by a third wave of synthetic opioid overdoses. These synthetic opioids, most particularly fentanyl, often arrive in the hands of users unknowingly and can prove deadly despite administration of the opioid antagonist naloxone. Public health organizations and harm reduction services require real-time, comprehensive data to allow for the revision and adaptation of intervention and prevention strategies. Similarly, policy makers and law enforcement agencies need data synchronous to current drug-use trends to quickly adapt drug legislation. Prior to the implementation of this syringe surveillance project, all drug-trend data was compiled from analysis of syringes from fatal overdose cases (death investigations) and law enforcement seizures of suspected controlled dangerous substances. While data from death investigation syringes is beneficial in supporting medicolegal death investigations, the necessary information arrives after a casualty has occurred. Likewise, analysis of seized evidence can provide insight into the District of Columbia's drug supply, but lacks confirmed usage trends by current IV drug users.

This presentation will impact the forensic science community by demonstrating how the analysis of drug residue will equip laboratories with essential supportive data, while illustrating much needed current trends in injection drug use in Washington, DC. In cooperation with three local needle-exchange programs, anonymously donated syringes were collected on a weekly basis. Syringes were collected individually in sharps safety tubes and geotagged with the collection site locations. The analysis of residual content in syringes was accomplished by performing a methanolic extraction prior to filtering, then Gas Chromatography/Mass Spectrometry (GC/MS). Batches were prepared and run bi-weekly, with time from exchange to analysis estimated between one to seven days. Geospatial analysis was performed using ArcGIS® Pro™, highlighting the prevalence of specific controlled dangerous substances. Preliminary data suggests a higher occurrence of methamphetamine used intravenously than was reflected in previously obtained data from death investigation syringes alone. Eutylone, a synthetic cathinone, has been observed for the first time in the residual content from multiple syringes. Additionally, an emerging trend of xylazine has been observed as a common fentanyl adulterant. Other preliminary data notes the high prevalence of fentanyl, both in combination with other opioids and alone, while simultaneously reflecting low incidence of unadulterated heroin.

Long-term implementation of this syringe surveillance program can offer a robust monitoring system of drug trends in Washington, DC, by combining with multiple laboratory data streams. This provides new intelligence on IV drug-user practices and emerging dangerous substances. Such knowledge is invaluable to users, public health officials, medical providers, emergency responders, and law enforcement agencies.

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### Needle Exchange, Syringes, Opioids

## B22 Method Validation and Detection of Adulterants in 1,031 Seized Drug Exhibits by High Resolution Mass Spectrometry

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**Learning Overview:** After attending this presentation, attendees will be able to describe current trends in the number and variety of adulterants encountered in seized material and implement an efficient workflow for the analysis of drugs of abuse and adulterants in seized material on a high-resolution mass spectrometry platform.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about a new and rapid methodology to screen for a wide range of cutting agents in seized materials. Also, attendees will have information about the prevalence of adulterants, many of which have significant toxicity, in seized drug evidence from several United States state jurisdictions.

Cutting agents, classified as diluents (pharmacologically inactive; e.g., sugars) and adulterants (pharmacologically active; e.g., phenacetin), are commonly used to increase profits. Cutting agents themselves as well as mixtures and ratios are constantly changing over time, which can increase risks to the user's health as a result of their side effects. Most laboratories in the United States report only controlled substances on the Drug Enforcement Administration (DEA) list (Schedules I to IV) or per their state regulations, which leads to under-reporting of other substances (adulterants) that may contribute to the adverse effect profile of illicit drug use. The goal of this work was to develop and validate a screening method to analyze 54 adulterants and scheduled drugs in seized drug evidence.

Analysis was performed on a VANQUISH™ Ultra High-Performance Liquid Chromatography (UHPLC) coupled to a Q-Exactive™ Orbitrap™/Mass Spectrometry (MS). The UHPLC was operated using a reverse-phase gradient using 0.1% formic acid in ultrapure water (MPA series) and 0.1% formic acid in acetonitrile (MPB series) for chromatographic separation on an Accucore™ C18+ analytical column (100mm x 2.1mm, 1.5µm). The UHPLC gradient initial conditions of 95A:5B changing to 5A:95B (3mins, hold till 3.5mins) and return to initial conditions and hold for 2.5mins at a flow rate of 0.3mL/min for a total run time of six minutes. Precursor ions were acquired by full MS scan (50–750m/z) via positive electrospray ionization. Precursor isolation was performed using dd-MS<sup>2</sup>. Fragmentation was achieved using stepped collision energy of 20, 40, and 80eV. Data processing was performed using Trace Finder™ software with an Extracted Ion Chromatogram (XIC) list containing 54 compounds, including fragments and accurate mass library.

A fit-for-purpose validation was performed for this method including precision, limit-of-detection, and carryover experiments following United Nations Office on Drugs and Crime (UNODC) and Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines. The method was successfully validated for 52 substances. Gabapentin and carisoprodol failed to be properly detected and identified following the processing parameters due to coelution with theophylline, codeine, and alprazolam. Adjustments in the processing criteria rules were made in case of detection of one or more compounds mentioned above in the same sample. The method was free from carryover up to the concentration of 1mg/mL. The limit of detection was defined for all compounds at 100ng/mL.

De-identified evaporated autosampler vials from seized drug exhibits collected in Pennsylvania, North Carolina, Texas, Virginia, New York, Georgia, and Maryland were received and linked to an in-house identifying number, date of receipt by the originating lab, and the zip code or county of origin. Prior to analysis, the samples were reconstituted in 1,000µL of mobile phase. The following data is representative of 1,031 samples. Overall, 51 substances were detected in the samples, and the most prevalent toxic adulterants found were lidocaine (12.97%), levamisole (10.84%), quinine/quinidine (10.45%), caffeine (10.16%), and procaine (8.71%). Fentanyl and heroin were mostly adulterated with quinine/quinidine and lidocaine, while cocaine was mostly adulterated with levamisole (56.2% of the samples) and phenacetin (28.4% of the samples). Tetrahydrocannabinol (THC) and methamphetamine were less adulterated in general, only 7.5% and 15.8 of those samples contained adulterants, respectively, while fentanyl, heroin, and cocaine samples were adulterated 89.3%, 73.2%, and 93.2% of the time, respectively.

Knowledge about cutting agents is commonly overlooked either because they are not detected or not reported. This leads to a lack of information that could be useful for management of acute intoxications in hospitals or criminal investigations or in helping in the identification of routes of trafficking. Common reporting practices, however, frequently do not provide information about the prevalence of these toxic adulterants.

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### Seized Drugs, Cutting Agents, Toxicity

## B23 Assessing the Performance of Fingerprint Laboratories Through Collaborative Exercises and Proficiency Tests: A Discussion Based on the European Experience

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**Learning Overview:** The goal of this presentation is to demonstrate which methodologies can be successfully used to evaluate the performance of latent print visualization and comparison laboratories while assessing the competence and the performance of the latent print experts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing the results of the performances of the fingerprint laboratories in Europe, assessed between 2004 and 2019 and collected through collaborative exercises and proficiency tests, organized by the European Network of Forensic Science Institutes (ENFSI) Fingerprint Working Group. Error rates and methods to calculate them will be widely discussed, presenting not only a realistic picture of state-of-the-art of the profession, but also a reliable methodology to assess its performance.

Since 2004, the ENFSI Fingerprint Working Group has started collaborative exercises and proficiency tests, in order to establish a robust methodology to measure the performance of its laboratory members. A large variety of test types have been designed and proposed, and results have been collected and discussed, giving some clear indications on the European Forensic Science Providers performances in latent print detection and identification tasks, showing a great difference between the results of the laboratories and identifying some weaknesses. Moreover, through the years, the critical review of the test types and the objective assessment of their inherent difficulties allowed determination of a robust methodology that can measure the capability of a laboratory (or of a fingerprint expert) to perform regarding a specific task.

The knowledge gained through the decades established, in 2016, a permanent Advisory Group within the ENFSI Fingerprint Working Group, with the task of managing an on-going testing program in order to ensure continuity and to plan testing in a way that meets the strategic objectives of ENFSI.

From the visualization perspective, a series of collaborative exercises have been carried out, some of them considered complex items constituted by different surfaces; some others tackled the issues of difficult surfaces, others went back to the basics, assessing the performance of the European laboratories toward very specific and simple tasks, which had failed in previous years' tests. An overview of the test designs will show the technical difficulties of the realization of consistent samples, and the presentation the results observed will provide a realistic description of the technical capabilities of fingerprint detection laboratories.

On the comparison side, during the evaluation of proficiency or competency tests, the attention is usually focused on the error rates. What are the different types of errors and what is their impact to the criminal justice system? Most of the time, the interest of the forensic stakeholders has been driven by the latent print expert's performance, while the most interesting parameter for the trier of facts is the forensic science provider's performance.

Thus, the aforementioned aspects will be thoroughly discussed, while enlightening attendees on how error rates are calculated, with an eye on what was reported in the 2016 President's Council of Advisors on Science and Technology (PCAST) Report.<sup>1</sup> Red flags will be disclosed to the audience and future corrective actions will be proposed. Moreover, interesting outcomes of the collaborative exercises and proficiency tests carried out within the ENFSI Fingerprint Working Group will be presented, thus demonstrating how the critical review of the results could improve the quality of the daily activities.

### Reference(s):

- <sup>1</sup> 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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### Latent Prints Labs, Performances, Error Rates

## B24 Latent Print Quality in Blind Proficiency Testing: Using Quality Metrics to Examine Laboratory Performance

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**Learning Overview:** This goal of this presentation is to educate attendees about preliminary findings from a Blind Quality Control (BQC) program in the Latent Prints section at the Houston Forensic Science Center. Additionally, this presentation will detail the quality of latent prints examined as part of this program and explore how this information can be used to improve similar programs as well as routine casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating a blind, latent print quality control program to demonstrate how such programs, supplemented with quality metrics, can improve forensic case work. Attendees will be encouraged to reflect upon their practice and consider the feasibility of incorporating BQC programs into their labs and quality metrics into routine casework.

**Introduction:** In 2015, the Houston Forensic Science Center adopted the recommendations detailed in the 2009 National Academy of Sciences (NAS) Report for blind proficiency testing by implementing a BQC program.<sup>1,2</sup> The intent of the BQC program is to supplement open proficiency tests that are required for accreditation and provide a way to monitor the entire quality management system from evidence submission to reporting of results. The program was initiated in the Latent Prints section in November 2017, and lab personnel routinely track case outcomes and print quality associated with all blind cases.

The current study sought to: (1) describe preliminary results from a blind testing program within a latent print unit of a crime laboratory, (2) examine the quality of submitted prints via quality metrics software, and (3) examine the potential association between latent print quality and resulting sufficiency determinations and identification conclusions.

Since its implementation, the quality division within the laboratory has developed and inserted 290 blind cases/requests for analysis into the latent print comparison unit. Print images for 144 of these cases were obtained for the present study. In total, examiners reviewed 376 latent prints as part of the 144 cases. Most blind cases involved only one latent print; however, some involved as many as 13 latent prints. The majority of latent prints were fingerprints (94.3%) or palm prints (4.9%).

Of the 376 prints, 92.0% were determined to be of sufficient quality to enter into the Automated Fingerprint Identification System (AFIS), and 6.1% were determined to be of comparative value, but insufficient quality to enter into AFIS. Very few prints were determined to be of no comparative value (1.6%).

Most prints were submitted with the true source present in AFIS (80.3%;  $n = 302$ ). Based on ground truth, examiners arrived at the “correct” conclusion in about half of all submitted prints (51.1%). Specifically, 33.0% of all latent prints were correctly associated with their source and 18.1% were correctly excluded from erroneous associations. There were no instances of false positive errors (i.e., associating a latent print with the wrong source print), but 41.0% of all prints were concluded to have no association with other prints despite the true source being in AFIS. Of course, there are multiple reasons why prints might not be associated with their source, including poor print quality, limitations of AFIS algorithms, or examiner error. There were only two occasions when the source candidate was listed in AFIS results and the examiner concluded that no association existed.

The quality of all latent print images was assessed using the Federal Bureau of Investigation’s (FBI) Universal Latent Workstation Latent Quality Metrics (LQMetrics) software.<sup>3</sup> This presentation will present detailed findings, but for clarity within this proposal, the quality of all prints will be categorized according to the traditional *Good*, *Bad*, and *Ugly* criteria defined by LQMetrics software. Put simply, print quality was associated with sufficiency determinations and, to a greater extent, ultimate conclusions. Prints belonging to the *Ugly* quality category were 0.88 times less likely to be determined to be of sufficient quality for AFIS entry and were more likely to be of no comparative value. Regarding accuracy, *Good* latent prints were 2.18 times more likely to result in a correct conclusion than were *Ugly* prints, whereas *Ugly* prints were 3.71 times more likely to result in inconclusive conclusions (i.e., no AFIS searches) than were *Good* prints. The accuracy of conclusions regarding *Bad* prints fell between *Good* and *Ugly* prints. This presentation will address how the use of quality metrics can improve the effectiveness of blind testing programs and routine casework.

### 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. <https://www.ncjrs.gov/pdffiles1/nij/grants/228091.pdf>.
2. Hundl, C., M. Neuman, A. Rairden, P. Rearden, and P. Stout. Implementation of a Blind Quality Control Program in a Forensic Laboratory. *Journal of Forensic Sciences* 65, (2019): 814-822.
3. *Federal Bureau of Investigation (FBI) Universal Latent Workstation (ULW) LQMetrics User Guide*. 2015.

### Blind Proficiency Testing, Latent Prints, Quality Metrics



## B25 A Non-Destructive Collection of Latent Fingerprints Using Gellifters® and GLScan®

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**Learning Overview:** The goals of this presentation are to: (1) demonstrate the effectiveness of Gellifters® in conjunction with GLScan® to collect and visualize latent fingerprints of value on porous and semi-porous substrates, and (2) highlight the improved performance of Gellifters® as compared to the recovery of latent prints using alternate light sources.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, specifically crime scene and evidence technicians and latent print examiners, by providing a method using Gellifters® and GLScan® that improves the performance of non-destructive collection of latent prints on porous and semi-porous substrates.

Non-destructive methods for latent fingerprint collection are highly sought after. In the current latent print development process, the furthest an examiner can process a piece of porous evidence non-destructively is up to visual inspection with white light and alternate light sources. However, there are a limited amount of latent prints that naturally fluoresce when viewed with alternate light sources. One way to increase the number of latent fingerprints developed without destroying the evidence could be with the use of Gellifters®. These gelatin lifts consist of a jet-black low-tack adhesive layer with a high-gloss finish and are typically used to collect latent shoeprints from various surfaces.<sup>1</sup> Collection is performed by laying the Gellifters® on a surface, removing it, and then placing the lift onto a scanner known as the GLScan® that produces high-quality digital images for analysis.<sup>1</sup>

This experiment was designed to determine if: (1) suitable latent prints for comparison can be captured on porous and semi-porous substrates; (2) substrates remain intact post-lifting; (3) GLScan® provides better results than currently used alternate light sources to detect latent prints; and (4) suitable latent prints for comparison can be captured after aging. To test these, prints were left on multiple porous and semi-porous substrates that were then collected using Gellifters® and scanned with GLScan® while also assessing if the substrate remained intact. Some of the samples from this study were stored for one month to assess whether prints of value could be collected after aging. In a later experiment, the capabilities of Gellifters® were compared to alternate light sources to determine the relative effectiveness of each. It was determined that latent fingerprints of value can be collected on both porous and semi-porous substrates even after being aged, while keeping the substrate intact. The comparison between alternate light sources and GLScan® showed that GLScan® performed better overall than alternate light sources. More research needs to be conducted; however, Gellifters® in combination with GLScan® appear to be a viable non-destructive collection method for field use.

### Reference(s):

- <sup>1</sup> BVDA Gellifters®. *Lift your evidence to the highest level*. 2012.

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### GLScan®, Gellifters®, Latent Prints

## **B26 A Statistical Method for Evaluating the Reliability of Identification of Randomly Acquired Characteristics (RACs) in Footwear Impression Evidence**

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**Learning Overview:** After attending this presentation, attendees will have learned about a statistical approach to quantifying the reliability of shoe print examinations, specifically the consistency with which RACs are identified by examiners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering the statistical foundation of a method for evaluating the performance of footwear examiners in identifying RACs. This is an important contribution to learning about the reliability of footwear examinations.

There has recently been increased attention on the reliability and validity of methods used in the analysis of forensic evidence. Black box studies, studies in which questioned-known evidence pairs for which the underlying truth is known are analyzed by forensic examiners, have emerged as a powerful tool to learn about intra-examiner reliability, inter-examiner reliability, and validity. Results of such studies can be complemented by additional studies that focus on performance of examiners on different aspects of the examination. This presentation focuses on the development of a statistical approach to assessing the performance of examiners in identifying RACs on footwear impressions.

Footwear examiners are asked to compare a footwear impression found at a crime scene (sometimes known as the questioned impression) with an impression from one or more suspect (known) shoes. The first step in this process is to rule out any shoes that do not match the basic characteristics (size or tread pattern) of the questioned impressions. Examiners next consider wear. A critical part of the examination process is the identification and examination of RACs, markings that indicate scratches or holes that have formed on the bottom of shoe soles as the shoe is worn. If the questioned and known impressions have RACs in the same location, then this is evidence that supports the hypothesis that the two impressions have a common source. Identifying RACs takes a great deal of training and, in many cases, is a difficult and time-consuming process. Footwear examiners record the location, type (holes, scratches, etc.), and other key attributes of each RAC. The proficiency tests that exist are designed to evaluate examiner performance when it comes to their ability to correctly match a questioned impression to a putative source. While RACs are considered in this decision-making process, there has been little research done in evaluating the performance and reliability of footwear examiners when it comes to the identification of RACs on a footwear impression. This performance assessment is complicated by the lack of ground truth of RAC occurrence.

The method described in this presentation is a first step in estimating the performance of examiners in identifying RACs examinations. It is based on a probability model that assumes there are repeated annotations of the same impression either by a single examiner or by multiple examiners. Importantly, the approach does not require that there be an *a priori* definition of ground truth. The probability model builds on an approach developed in the context of brain imaging.<sup>1</sup> Data from a pilot study conducted by the Division of Identification and Forensic Science of the Israel Police (DIFS) allows for the estimation of performance because multiple student trainees identified RACs on the same footwear impression and each examiner examined multiple shoes. Using this data, performance parameters were defined as the ability of the examiner to determine whether or not RACs appear on the impression. Further, the model can incorporate information from multiple footwear impressions. Preliminary results have indicated this approach is a step in the right direction regarding estimation of the performance of footwear examiners.

### **Reference(s):**

- <sup>1</sup> Simon K. Warfield, Kelly H. Zou, and William M. Wells. Simultaneous Truth and Performance Level Estimation (STAPLE): An Algorithm for Validation of Image Segmentation. *IEEE Trans Med Imaging* 23 no. 7 (2004): 903-921.

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### **Performance Assessment, Examiner Reliability, Randomly Acquired Characteristics**

## **B27 A Tisket, A Tasket, Tribolium in Your Basket: The Development of a New Approach for the Forensic Detection of Stored-Product Insect Pests Using Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART®-HRMS) and Chemometrics**

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**Learning Overview:** After attending this presentation, attendees will understand the use of DART®-HRMS as a screening device to rapidly test agricultural products for the presence of insect pests to determine if the products meet regulation standards.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding a new technique that investigators can use to detect insect infestation in agricultural products while determining fault in cases of health and safety violations.

Insect pests infesting agricultural products leave behind chemical markers which, through a combination of DART®-HRMS and statistical analysis can be used to identify and quantify the infestation in the products.

Governing bodies such as the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) are charged with setting strict health and safety guidelines for food products in order to protect consumers. Failure to adhere to these regulations can result in the transmission of microbial contaminants and toxins to consumers, litigation and fines for food producers and manufacturers, and spark larger investigations into the food safety violations. The ability to accurately and rapidly detect insect presence and scale of infestation in agricultural products is therefore of the utmost importance to both regulating bodies and food producers and manufacturers. Unfortunately, conventional techniques used in stored-product forensic entomology generally require significant specialized expertise, are time-consuming and resource-intensive to implement, and exhibit variable accuracy in detecting the type and severity of insect infestation. Therefore, there remains a need for simple, rapid, and reliable techniques for the assessment of food pest infestation in order to establish liability in court cases revolving around food health and safety violations.

It is demonstrated here that a chemical approach to the testing of agricultural products could address many of the challenges presented by the conventional techniques stated above. In accordance with Locard's Exchange Principle, insect invaders in processed food products such as milled grain should transfer chemical biomarkers to their surrounding environment. Further, these species-specific insect biomarkers should lead to both identity determination of the invading species, as well as an estimation of the insect population present, through correlation of these factors with the quantity of the observed biomarkers. The unique capabilities of DART®-HRMS make it ideal for this type of rapid screening analysis. In a proof-of-principle study, a common agricultural pest, *Tribolium castaneum* (the red flour beetle), was used to deliberately infest all-purpose flour. These insects were introduced into five separate batches of flour, each in replicates of five, while an equal number of samples of non-infested flour were used as controls. The samples were analyzed over the course of several months by DART®-HRMS in positive-ion mode in order to monitor the appearance of insect-associated molecules as the length and size of the simulated infestation grew. The collected spectra were used to generate chemical profiles for the control and infested flours, which were then subjected to several methods of statistical analysis including Analysis of Variance (ANOVA) - Simultaneous Component Analysis (ASCA). This analysis highlighted several masses that enabled the differentiation of infested and non-infested flour. One of the masses,  $m/z$  137, was confirmed by Gas Chromatography/Mass Spectrometry (GC/MS) analysis to be a molecule frequently associated with red flour beetles: 2-Ethyl-1,4-Benzoquinone (EBQ).

Investigators of health and safety violations of agricultural products remain in need of a simple and reliable technique for the rapid detection and evaluation of insect infestations. The results of this study indicate the potential for DART®-HRMS to be used as a tool by these investigators to determine the scope and scale of insect infestation in processed agricultural products.

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**Forensic Entomology, DART®-HRMS, Chemometrics**

## B28 The Effect of Modifications to the Core Fentanyl Structure on the Observed Product Ion Spectra

Alia S. Hacker, BS\*, West Virginia University, Morgantown, WV 26505; J. Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX 77340; Glen P. Jackson, PhD, West Virginia University, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will understand how the abundance of diagnostic product ions can be used to predict the region of modification of novel Fentanyl-Related Compounds (FRCs). Attendees will also learn about how multistage Mass Spectrometry (MS<sup>n</sup>) can be used to determine differences in structures of isobaric product ions.

**Impact on the Forensic Science Community:** This presentation will benefit the forensic science community by providing a deeper understanding of spectral interpretation of fentanyl analogs in tandem mass spectrometry.

The central hypothesis is that by identifying trends or correlations between certain types of modification and the abundance of diagnostic product ions, analysts could use these trends to help determine the structure of novel FRCs from their mass spectra.

During a previous study, Davidson et al. found evidence for a novel R-group transfer in the Collision-Induced Dissociation (CID) spectra of protonated fentanyl analogs from an Electrospray Ionization (ESI) source.<sup>1</sup> Davidson et al. found that a product ion at  $m/z$  244 likely formed through the transfer of the propionaldehyde group of fentanyl from the aniline moiety to the piperidine nitrogen. The proposed mechanism involves a nucleophilic attack of the carbonyl carbon by the lone pair on the piperidine nitrogen atom. This transfer is followed by cleavage of the aniline ring and the formation of a double bond on the piperidine ring. Using MS<sup>3</sup> data, Davidson et al. also showed that the product ion at  $m/z$  244 readily loses cyclobutene (54 Da) to form a subsequent fragment at  $m/z$  190.

In a subsequent study, Davidson et al. observed the fragmentation behavior of fentanyl, fentanyl d5, and 14 other FRCs and found that only 11 of these compounds gave a product ion at  $m/z$  244 or its equivalent mass.<sup>2</sup> The current work focuses on a quantitative analysis of the previously reported spectra with the addition of selected fentanyl analogs that were expected to influence the R-group transfer in systematic ways.

Of the currently studied fentanyl analogs, the amide R-group transfer pathway contributes less than 1% of the abundance of product ion spectra, so this pathway is minor relative to other dominant pathways. However, quantitative comparisons of the R-group transfer pathways show that larger electron donating groups on the R2 (amide) region tend to enhance the abundance of the product ion at  $m/z$  244 relative to the core structure of fentanyl. Also, modifications at the R3 and R4 positions (the piperidine ring and alkyl chain, respectively) tend to encourage competitive pathways and hinder the abundance the R-group transfer. R1 modifications appear to have little effect overall on the abundance of the product ion at  $m/z$  244 or its equivalent. Modifications on the R5 position (phenyl ring) has a negligible effect on the abundance of the R-group transfer because this modification region is distant from the rearrangement region. The use of MS<sup>n</sup> allowed for the detection of these trends in the product ion spectra and an improved understanding of how diagnostic product ion abundances can be used to determine areas of modification on novel FRCs.

### Reference(s):

1. J. Tyler Davidson, Zachary J. Sasiene, Glen P. Jackson. The characterization of isobaric product ions of fentanyl using multi-stage mass spectrometry, high-resolution mass spectrometry and isotopic labeling. *Drug Test Anal* 2020, 12 (4), 496-503.
2. J. Tyler Davidson, Zachary J. Sasiene, Glen P. Jackson. The Influence of Chemical Modifications on the Fragmentation Behavior of Fentanyl and Fentanyl-Related Compounds in Electrospray Ionization Tandem Mass Spectrometry *Drug Test. Anal.* 2020, 12 (7), 957-967.

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### Fentanyl-Related Compounds, Predictive Identification Method, Tandem Mass Spectrometry



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## **B29 Defining and Explaining Comparative Spectral Differences Within the Organization of Scientific Area Committee's (OSAC's) Trace Materials Standards**

*Diana M. Wright, PhD\*, Federal Bureau of Investigation Laboratory Division, Quantico, VA 22135*

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**Learning Overview:** The goal of this presentation is to educate and inform the trace evidence community on a consensus approach to describing visual evaluation of comparative spectral data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing information that has been in discussion in the OSAC Trace Materials Subcommittee as to an appropriate and more uniform approach to evaluating and recording the results and interpretation of comparative spectral data analysis.

In this presentation, attendees will learn why the Trace Materials Subcommittee of OSAC has begun to implement a new term to describe differences observed between items being compared. This term is “exclusionary difference” and is defined as “a difference in a feature or property between compared items that is substantial enough to conclude that they did not originate from the same source.” Included with this definition are two notes that are used to further clarify the inclusion of both statistical and non-statistical evaluations within the context of this term. The first note reads as: “An exclusionary difference is statistically supported when an appropriate statistical analysis shows a result outside the range of what usually occurs when the items originate from the same source.” The second note is: “When a statistical analysis is not suitable, an exclusionary difference can be determined by expert judgment.”

The use of the word “exclusion(ary)” is intended to align with the legal community’s understanding of this term: to indicate that two or more items could not share a common source. The two notes were added so that it is clear that regardless of how the exclusion is made, the expert opinion is an elimination of common origin.

This term is being included throughout all current draft documents originating from the Trace Materials Subcommittee and will be used to replace the term “meaningful difference” in previously issued documents as well. It is expected to also be implemented in other OSAC disciplines, where applicable.

It is recognized that defining an exclusion is only one piece of the interpretation guidance necessary to compare data. Most trace evidence is still evaluated exclusively or in part using visual comparison of the graphical data. To this end, the Trace Materials Subcommittee has developed and approved a template of language to describe how spectral overlays are evaluated for distinguishability. Regardless of the methodology used to collect spectral data, the process is being inserted into the spectral evaluation section of Trace Materials documents to explain how spectral comparisons are assessed and how that data fits into the overarching analytical scheme used to examine and compare materials.

This language and examples will be presented to demonstrate how the terms and their definitions appear in the American Society for Testing and Materials (ASTM) standards originating from the Trace Materials OSAC subcommittee. It is hoped that more consistent language across trace evidence technique-based documents will provide clarity as to the strengths and limitations of comparative trace examinations.

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### **OSAC, Trace Materials, Comparison Language**

## B30 The Development of a Multivariate Mass Spectral Algorithm for the Identification of Seized Drugs

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**Learning Overview:** After attending this presentation, attendees will understand how correlated relationships between mass spectral ion abundances can be used to discriminate between compounds with seemingly indistinguishable electron ionization mass spectra.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a flexible statistical model that can minimize the risk of false positive and false negative drug classifications in seized drug analyses that use Electron Ionization/Mass Spectrometry (EI/MS) as a basis for identification.

The central hypothesis is that a multivariate algorithm that takes advantage of the innate correlations between ion abundances of replicate spectra can make more accurate predictions than existing compound identification algorithms.

Most search algorithms for EI/MS data make use of a “static” exemplar approach when making unknown identifications. Although different vendors’ algorithms vary in their approach, they all compare unknown spectra to discrete, fixed spectra of standards in a library. However, the inter-day or inter-laboratory variance in the abundance of each fragment in a spectrum is known to vary by  $\pm 20\%$ , so compounds with somewhat similar EI-mass spectra, like many fentanyl analogs, can be difficult to be confidently distinguished using only the EI spectral comparison. The multivariate algorithm discussed in this presentation takes a more informed approach. It uses an algorithm to effectively interpolate between replicate spectra and provide a continuously variable model of ion abundances for each compound in the database. The model explains most of the variance in replicate mass spectra and enables very confident mass spectral identifications.

A library of spectra for fentanyl analogs was extracted from Gas Chromatography/Electron Ionization/Mass Spectrometer (GC/EI/MS) data by extracting every mass spectrum across the eluting chromatographic peaks of interest. Each chromatogram, therefore, provides seven to ten replicate spectra of various intensities. The 15 most abundant ions were extracted, and the abbreviated spectra were randomly divided into calibration and validation sets. Fifteen General Linear Regression Models (GLMs) were built for each compound by sequentially using each ion’s abundance as the dependent variable and the abundance of the remaining 14 ions as the independent variables. In each model, variables were added stepwise until there were no significant improvements in the predictions, which resulted in each model containing four to eight variables. The 15 GLM models for each compound were then used to predict 15 ion abundances in a variety of known positives and known negatives. The predicted abundances were compared to the measured abundances using a variety of similarity and dissimilarity metrics like the Pearson Product-Moment Correlation (PPMC). Each method of similarity scoring was used as a binary classifier to determine True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN) rates over a range of threshold values. These classifications were then used to plot Receiver Operating Characteristic (ROC) curves.

PPMC values between measured and predicted spectra of known positives in the fentanyl models exceeded 0.9939 and 0.9977 for the calibration and validation sets, respectively. Known negatives in the validation set typically had PPMC values smaller than the smallest PPMCs for known positives, which resulted in Areas Under the Curve (AUC) of 1 in the ROC plots for binary classification. The residuals in predictions were typically improved by a factor of three or more using the dynamic model relative to the traditional, static approach.

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### General Linear Model, Mass Spectrometry, Fentanyl Analogs

## B31 Mexican Methamphetamine Manufacturing Investigations

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**Learning Overview:** This presentation will inform attendees on the analytical tools and techniques used to investigate multi-step, large-scale synthesis of methamphetamine in Mexico. Attendees will be able to identify these tools/techniques used to investigate complex synthetic schemes associated with methamphetamine synthesis and how these forensic investigations can enhance clandestine laboratory investigations, both domestically and internationally.

**Impact on the Forensic Science Community:** This presentation will impact the forensic drug chemistry community by presenting an example of multiple disciplines of chemistry, deployable instrumentation, and communications to enhance international clandestine laboratory investigations. Considering that the United States has previously seen fluctuations in the occurrence of clandestine laboratories, it is essential that this generation of forensic drug chemists establish context so as to be prepared for the next set of synthetic drug laboratories in their respective areas of responsibility.

A significant portion of forensic drug chemistry is focused on the analytical chemistry discipline. This is not a surprise considering that the charge that most forensic drug chemists have is related to the in-house analysis of controlled substances in their finished state. The investigation of clandestine laboratories was once a substantive portion of forensic drug chemistry that tended to draw on the organic chemistry discipline in addition to analytical chemistry. Per this study's observation, it appears that fewer and fewer forensic drug chemists are called upon to investigate clandestine laboratories. Some of this trend can be attributed to the growing portions of United States-seized methamphetamine being synthesized in Mexico and the commensurate drop in the number of domestic methamphetamine clandestine laboratories. Consequently, many forensic drug chemists may lack some of the essential skills in the investigation of clandestine laboratories.

Mexican methamphetamine-manufacturing drug trafficking organizations use a vast array of precursors leading to a very complicated synthetic scheme. There are a vast number of known routes of synthesis just for methamphetamine, let alone other synthetic or semi-synthetic drugs, that require constant attention. Furthermore, these schemes challenge chemists not only in their scope but also in the field-detection/presumptive indications. Techniques that provide excellent discrimination and presumptive indications include Raman and infrared spectrophotometry. These techniques are portable and ruggedized for use on-scene

Finally, communicating a very complicated situation to investigators and prosecutors is absolutely essential despite chemistry's esoteric language and any number of naming conventions employed to identify commonly traded chemicals used for illicit purposes. This communication demands that the forensic drug chemist stay abreast of newly encountered precursor materials as well as how they impact known production/synthesis methods and yields. Consequently, this ever-evolving information must then be conveyed to investigators and prosecutors in plain language.

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### Clandestine Laboratory, Drug, Deployable Instrumentation

## B32 The Development of RED-BLEU: A UV/Vis Assay Following Colorimetric Detection of EDTA

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**Learning Overview:** After attending this presentation, attendees will be familiar with a new Ultraviolet/Visible (UV/Vis) spectroscopy component for the presumptive detection of disodium Ethylenediaminetetraacetic Acid (EDTA) in blood samples. This test is designed to accompany the previously reported colorimetric assay—Reverse EDTA Detection (RED)—with the main purposes of reducing subjectivity and false positives/negatives, as well as quantifying EDTA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by supplementing previous research to bring a new presumptive method to the forefront that can indicate the presence of EDTA in blood samples, thereby aiding in the detection of planted blood evidence.

The detection of EDTA can help support or refute allegations of planted blood evidence because it is not a natural component of blood.<sup>1</sup> This research group has previously developed the RED colorimetric test for EDTA detection using Eriochrome® Black T (EBT), and this presentation will focus on the development of an extension of that method using UV/Vis to yield the Reverse EDTA Detection in Blood using EBT and UV/Vis (RED-BLEU) test.<sup>2,3</sup> To test this, a variety of samples with and without EDTA were subjected to the RED test, followed by analysis of 2µL of the tested sample on the NanoDrop™ 2000.

A multifaceted approach was taken to develop the UV/Vis portion of the assay: Wavelengths of interest were identified to corroborate the observed color change and EDTA quantification, the latter of which also required the development of a standard curve. To identify wavelengths of interest, samples prepared in triplicate concentrations of 0.1, 0.5, 1, and 10mg/mL EDTA were tested with the colorimetric assay, then processed in duplicate on the NanoDrop™. Absorbance values from these samples were compared to those of five blanks (processed in triplicate) to identify wavelengths with significant differences in absorbance. From this, wavelengths 192–196nm were identified as possible candidates for EDTA quantification, while 520–540nm, 640nm, and 650nm were noted as wavelengths of interest for an objective determination of color change.<sup>4,5</sup> Samples containing EDTA yielded a blue color change with a UV/Vis spectrum consisting of a discernable peak ~192–196nm and a flat baseline indistinguishable from blanks for the remainder of the spectrum. Conversely, samples without EDTA produced a pink color change with a UV/Vis spectrum exhibiting absorbance values elevated from the baseline ~520–540nm and less than the baseline at ~640nm and 650nm; however, they lacked the 192–196nm peak seen in samples with EDTA. Thus, the absorbance values at these wavelengths can be used to objectively determine whether EDTA is present.

The development of a standard curve to quantify the amount of EDTA present required multiple considerations: selection of wavelength, standard concentrations, and line of best fit, as well as identifying the impact (if any) of wait time. Wait time was assessed at two points: (1) 10, 30, or 60 minutes between adding the buffer and EBT indicator, followed by immediate UV/Vis testing; or (2) 10 or 30 minutes between the RED test and UV/Vis testing. Each criterion was tested in triplicate and was eliminated if the resulting standard curve had an  $R^2 < 0.98$ . The best performing standard curve consisted of a linear trendline from absorbance values at 196nm and incorporated EDTA concentrations of 0.1, 0.5, 1, 3, 6, and 10mg/mL (processed in duplicate). Wait times of 10–30 minutes between addition of buffer and EBT indicator or 10 minutes between the RED test and UV/Vis analysis consistently yielded passing  $R^2$  values.

Once validated, the RED-BLEU assay for EDTA detection will provide all forensic laboratories with a quick, inexpensive presumptive test to detect planted blood evidence.

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### RED-BLEU, Blood, EDTA



## B33 Validating the Sexual Lubricant Database Using True Known and Unknown Samples for Forensic Analysis

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**Learning Overview:** The goal of this presentation is to outline the validation process of a sexual lubricant database. After attending this presentation, attendees will be informed of an established sexual lubricant database and its potential in the identification and classification of sexual lubricants.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a database that can be incorporated into sexual assault cases. This database contains reference lubricants analyzed on both spectroscopic and spectrometric instruments that are common for analyzing trace evidence.

Efforts to analyze, identify, and classify sexual lubricant samples in the unfortunate cases of sexual assaults have led to the development of a sexual lubricant database established by the National Center for Forensic Science. Currently, the database is a compilation of over 100 reference sexual lubricants of various marketing types. The database provides characterization and instrumental data, as well as classification models for unknown samples. Research has demonstrated that sexual lubricants can be classified into distinctive groups based on chemical composition, some of which are linked to specific marketing types based on the presence of unique compounds (e.g., personal hygiene projects, bottled lubricants or condom lubricants). Therefore, the samples contained in the database were analyzed to provide classification models that can be utilized in classifying unknown sexual assault samples.

All lubricant samples in this study were analyzed in their neat form using Fourier transform infrared spectroscopy and direct analysis in real time-high resolution mass spectrometry in the case when bulk evidence is submitted to the laboratory (e.g., condom wrappers). The samples were also extracted in methanol or hexane for analysis on gas chromatography/mass spectrometry. The extracts of lubricants were also necessary to simulate trace evidence scenarios, in the event that only a swab of a lubricant residue was submitted to the laboratory. Therefore, these extracts were also analyzed on the previously mentioned instruments to provide a comprehensive analysis of trace residues. The analysis of the data included supervised and unsupervised statistical techniques (e.g., hierarchical cluster analysis, Principal Component Analysis [PCA] and Linear Discriminant Analysis [LDA]). These statistical tools provided class assignments of the samples that were then used to calculate the percent error of the model using the projected classifications obtained in the LDA. Based on cross-validation of the parsed-out test set, all the models that were developed possessed classification error rates less than 5%. Further validation was essential for real-world scenarios.

The validity of the established method underwent two accuracy tests. First, 50 new samples were analyzed to evaluate the classification model using percent error. The theoretical “ground truth” classifications were determined by combining the data from the new 50 with the established classification model using PCA. Once these theoretical classes were determined, the projected classifications were then determined by using the 50 new samples as a test sample against the initial classification model. The theoretical and projected classifications defined the error rate of the classification model for a large set of new, “unknown” samples. Furthermore, a second validation method incorporated the use of real-world samples because reference samples can provide a chemical fingerprint but does not account for factors introduced during coitus. Lubricant residues were collected post-coitus from various points of contact on the sexual organs for analysis and subsequently subjected to classification using the initial classification model as well as the new classification model that incorporated the 50 new samples. This presentation expands on the evaluation of classifying known and unknown samples in the sexual lubricant database to operationalize it in real-world sexual assault cases.

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### Sexual Assault, Database, Trace Evidence

## B34 Assessing the Public's Opinion on the Use of Forensic Genetic Genealogy (FGG) in Criminal Investigations

*Claire L. Glynn, PhD\**, University of New Haven, West Haven, CT 06516; *Rachel Graziano, Johnston, RI 02919*; *Jessica Flynn, BS, West Haven, CT 06516*

**Learning Overview:** The goal of this presentation is to inform attendees on the relatively new and rapidly evolving method of FGG, including the public's opinion on the use of this method in criminal investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of gaining insight into the public's opinion on the use of FGG and ultimately educating the public on what this method entails.

FGG has emerged as a novel investigative tool and has rapidly gained much attention in recent years, in particular since April 2018, when it was announced that the Golden State Killer had been identified using this new method. While this was not the first case solved using this technique, the high-profile nature of the case certainly brought this method a lot of media attention, leading to a rapid increase in its interest and use in hundreds of cold case investigations. FGG broadens the field of forensic DNA analysis and combines genetic methods with traditional genealogical methods for building family trees. FGG differs from traditional forensic DNA profiling in both the type of DNA technology used and the DNA databases employed.

As this is a relatively new and a much more encompassing technique used as an investigative tool in criminal investigations, questions have arisen regarding its use, ethics, and privacy issues. The aim of this survey was to assess public opinion on the use of FGG in criminal investigations. A 32-question survey was created using the QualtricsXM<sup>®</sup> survey platform. The questions collected demographics of the respondents, followed by questions designed to assess their opinions on the use of FGG in criminal investigations. The survey received over 1,400 responses. The survey questions addressed the use of both public and private genetic genealogy databases. Private genetic genealogy databases refer to Direct-To-Consumer (DTC) DNA testing companies such as AncestryDNA<sup>®</sup>, 23andMe<sup>®</sup>, MyHeritage<sup>®</sup>, Family Tree DNA<sup>®</sup>, etc., from which consumers purchase kits and submit biological samples. The raw DNA data is available from these companies for users to download, which can then be uploaded to public genetic genealogy databases. Public genetic genealogy databases refer to free online databases such as GEDmatch<sup>®</sup>, to which users can voluntarily upload their raw DNA data to in order to broaden their search for genetic relatives.

Law enforcement agencies have utilized these public genetic genealogy databases (e.g., GEDmatch<sup>®</sup>) by creating a Single Nucleotide Polymorphism (SNP) profile of an unknown biological crime scene sample suspected to belong to the perpetrator, then uploading it to a public database in a search for shared DNA matches (i.e., genetic relatives). The investigators then build the family tree of the genetic relatives using traditional genealogy methods to resolve the identity of the unknown crime scene sample. The results of the survey show there is a willingness among the public to allow law enforcement to access public and private genetic genealogy databases, but generally only for major crimes. The results also show the opt-in/opt-out function for law enforcement access in both private and public genetic genealogy databases is favorable as it allows the user to control their own data. Interestingly, 57% of respondents believe a search warrant should be required. FGG has benefited hundreds of criminal investigations in recent years and may soon become routine practice for investigating major crimes as more crime laboratories begin utilizing FGG in their case work investigations. As the database sizes grow, so does the power of FGG.

### Forensic Genealogy, Forensic Genetic Genealogy, Investigative Genetic Genealogy

## B35 Whole Genome Sequencing (WGS) of Highly Degraded Samples for Forensic Genetic Genealogy and a Case Study

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**Learning Overview:** After attending this presentation, attendees will better understand the challenges for obtaining and analyzing DNA from rootless hair using WGS data. These WGS data are used to generate whole genome Single Nucleotide Polymorphism (SNP) profiles for purposes of genetic genealogy. The success and significance of this approach for Law Enforcement (LE) is outlined in a case study from Snohomish County, WA, which resulted in the successful identification of “Precious Jane Doe” after nearly 43 years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the value of rootless hair for use in DNA analyses, including investigative genetic genealogy. Rootless hair and other highly degraded samples are dominated by “ultrashort” DNA fragments (e.g., 30–60bp in length). These fragments are present in minute quantities and are often overwhelmed by the presence of exogenous, non-human DNA (e.g., microbial DNA). For these reasons, forensic samples, especially from cold cases, greatly benefit from the use of ancient DNA (aDNA) -based methods for DNA extraction and WGS library preparation designed to retain the shortest DNA fragments possible while maximizing the number of unique DNA molecules available for sequencing.

To date, DNA-based identification in forensics has typically relied on Polymerase Chain Reaction (PCR) -based assays targeting a handful of Short Tandem Repeat (STR) markers. While PCR is an extremely sensitive approach, the problem remains that the majority of STR amplicons are too long to amplify the ultrashort DNA contained in hair. A similar limitation is true for microarray technology, which can target millions of Single Nucleotide Polymorphisms (SNPs), and is the common method in Direct-to-Consumer (DTC) genetic tests. DTC tests as well as overall interest in genealogy have surged in popularity, accompanied by increasingly large databases containing human genotypes, or SNP profiles, used for finding relatives. Within the past few years, these databases have been repurposed for a new approach to human identification called forensic genetic genealogy, wherein relatives of unknown individuals are identified and used by LE as investigative leads. This approach has helped solve several high-profile cold cases such as the Golden State killer and “Buckskin Girl”. However, forensic samples may not contain the quantity or quality of recoverable DNA necessary for array-based genotype analysis. The solution is a WGS approach that is capable of producing genotypes from highly degraded samples in a format similar to DTC tests and compatible with genetic databases.

By applying aDNA techniques for extraction and library preparation to samples such as rootless hair or bone, it becomes possible to capture and sequence short, degraded fragments of DNA from both the nuclear and mitochondrial genome. The resulting WGS data are combined with an imputation approach to generate genotype files suitable for genetic genealogy investigation.

This approach was applied to rootless hair provided by the Snohomish County Sheriff's Office to identify human remains belonging to an unknown female recovered in August 1977. The remains were exhumed in 2008 for DNA testing. A partial genetic profile from the femur bone failed to yield a Combined DNA Index System (CODIS) match. Since then, at least four additional attempts to generate DNA data were unsuccessful. In 2019, hair samples sent to the University of California Santa Cruz/Astrea Forensics underwent DNA extraction using 5cm of a single rootless hair followed by library preparation, sequencing, and genotyping by imputation.

Results demonstrate the successful application of aDNA-like techniques for DNA extraction and library preparation on highly degraded forensic samples. From the library, 346 million sequence reads from the Jane Doe hair were used as input to generate a genotype containing 1,437,366 full autosomal SNP calls. The genotype file was submitted to the law enforcement agency and their genetic genealogists, and uploaded to GEDmatch®. By combining DNA and genealogical analysis, “Precious Jane Doe” was positively identified in 2020 as Lisa Roberts, 43 years after her remains were found.

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### Genetic Genealogy, WGS, Rootless Hair

## **B36 The Expansion of Genealogy Into Forensics: The Challenges of Converting a Commercial Industry Into a Forensic Industry**

*Marybeth J. Sciarretta, MS\*, DNA Labs International, Deerfield Beach, FL 33441; Jarrett P. Ross, BA\*, DNA Labs International, Deerfield Beach, FL 33441*

**Learning Overview:** This presentation will highlight the challenges that are being faced when forensic samples are used for Forensic Genetic Genealogy (FGG).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by being of interest to agencies looking to outsource their cases for genetic genealogy research.

There is an abundance of forensic cases that have had no current leads with autosomal Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) testing. Whether the case is seeking to identify an unidentified victim or a foreign profile that is believed to be the suspect, there are only so many possibilities for database searching STR profiles. A profile can be uploaded to the Combined DNA Index System (CODIS) database or the unknown profile can be compared against references standards that are submitted in the case. When those two avenues do not provide any possible connections, many agencies are now turning toward genealogy analysis. In many instances, homicide units are submitting cold cases for such analysis to private laboratories, as local and state government laboratories do not have the means for Single Nucleotide Polymorphism (SNP) testing, or on staff genealogists. Commercial laboratories and genealogists have faced new challenges by working with lower quantities of DNA, samples with degradation, and data with varying call-rates.

Private laboratories that perform SNP testing have only recently started analyzing samples for forensic applications. Typically, these laboratories perform commercial assays to assist with clinical diagnostics, genetic research, and relationship testing. Samples that are frequently analyzed include whole blood, buccal swabs, and frozen tissue. Unfortunately, forensic samples do not always result in such robust quantities of DNA. Many unidentified victim cases involve bone samples, which can either be degraded, have poor sample quality, or yield insufficient DNA. Lower quantities of human DNA and degradation issues are probably the biggest challenge facing forensic laboratories interested in genealogy. As the quantity and quality of the DNA coincide with the call-rate, the eligibility of a sample for upload into a database is affected.

When a forensic sample yields sufficient DNA for genetic genealogy, it is often still a degraded sample, which can cause issues for the genetic genealogy research. In genetic genealogy, the relationship between two genetic matches is estimated based on the shared DNA, which is measured using centimorgans (cM). When dealing with a degraded forensic sample, there is less DNA to match against others in the database, and this may cause certain matches to seem more distant due to less total shared cM and smaller segments of shared cM. This issue can also be exacerbated if the forensic sample came from someone who descends from an endogamous population. Techniques using chromosome mapping (a.k.a. DNA painting) are being developed to help mitigate these problems, but if successful, they still would not be able to completely resolve the issues resulting from degradation.

Another challenge with FGG, also referred to as Investigative Genetic Genealogy (IGG), is contacting genetic matches or other genealogists about the case. Due to the sensitive nature of FGG, labs do not directly contact any genetic matches or associated genealogists but instead pass along the information to law enforcement so they can make the contact through the appropriate channels. In traditional genetic genealogy, it is common to not only directly contact genetic matches or the associated genealogists but to also seek help through various public genealogy forums.

There are several steps between the time a sample is submitted for genetic genealogy analysis and the time the genetic genealogy research occurs. Some of these steps include a review of the questioned sample, outsourcing to a laboratory to perform the SNP testing, and uploading the results to one or more databases. Moving this entire process in-house may alleviate some of the challenges that are currently being tackled. This will also aid in streamlining the process by having analysts in-house review the submissions, sequence the data, and work continuously with a forensic genealogist. As this previously commercial industry further progresses into the forensic world, innovation will streamline the process further making FGG indispensable to cold case detectives.

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### **Forensic Genetic Genealogy (FGG), Single Nucleotide Polymorphism, Cold Case**

## B37 Alleles With Dissimilar Frequencies Between Ethnic Populations Increase the Uncertainty in DNA Mixture Interpretation

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**Learning Overview:** The goal of this presentation is to communicate this study's results after evaluating the impact of assigning different allele frequency databases to contributors of two-person mixtures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that Likelihood Ratios (LRs) produced from the current practice may be non-conservative when mixtures comprise alleles with substantially dissimilar frequencies between populations.

The current practice in the statistical analysis of mixed DNA profiles is to compute LR's assuming the same population database for each contributor. This practice does not consider that mixtures may comprise individuals best represented from different populations. LR's reflect more than the comparisons to a Person Of Interest (POI); they also consider the genotypes proposed for any unknown contributors. When the genotypes proposed for contributors favor different populations, it is possible for outcomes to be non-conservative. Although past studies have suggested that the impact of this uncertainty is minor, the impact of alleles with large differences in frequencies between relevant populations has not been considered. For example, the Harris County Institute of Forensic Sciences calculates LR's using three Federal Bureau of Investigation (FBI) populations (African American/Bahamian/Jamaican, Caucasian, and Southwest Hispanic populations), which include alleles showing substantial differences in frequencies; some alleles have frequencies ranging 1%–7.5% in one population while having zero observations in another. It was expected that LR's would be increased when rare alleles are present in mixtures.

Mixtures were deconvoluted using the probabilistic genotyping system STRmix™. Random non-contributor profiles producing LR's greater than 100 were identified using propositions: (H<sub>1</sub>) the DNA originated from the POI of a first population and an unknown contributor of a second population; and (H<sub>2</sub>) the DNA originated from an unknown contributor of the first population and an unknown contributor of the second population. Same- and different-population LR's were calculated based on the methods of STRmix™. The methods were adapted for calculating different-population LR's by considering zero coancestry between alleles sampled under different populations. The populations considered were the FBI African American/Bahamian/Jamaican, Caucasian, and Southwest Hispanic populations. Nine sub-source LR's were produced for each non-contributor profile. The most conservative same-population LR was compared to the most conservative LR over all nine outcomes. The impact of specific alleles having substantially dissimilar frequencies between populations was evaluated. Candidate alleles were those having at least about 100-times greater posterior mean frequency in one population over another.

Comparisons of 7,980,000 were made between two-person mixtures and random non-contributor profiles. Mixtures lacking candidate alleles showed a wide variation in outcomes resulting from considering different populations: 7.1% of non-contributor LR's were reduced by at least 90% in magnitude (largest observed was 97.0%), 71.2% were reduced by 10%–90%, and 21.7% were reduced by less than 10% or unchanged. The presence of candidate alleles decreased the proportion of LR's that were reduced by 10%–90%: the proportions for mixtures having one or two candidate alleles were 26.8% and 5.4%, respectively. This was accompanied by increases in the proportions of LR's that were reduced by at least 90% (58.5% and 21.6% for mixtures comprising one or two candidate alleles, respectively) or less than 10% (14.6% and 73.0%, respectively). Several LR's were reduced by at least 99%; the proportions for mixtures comprising one or two candidate alleles were 9.8% and 16.2%, respectively.

When mixtures comprised a single candidate allele, the proportion of LR's reduced by one or more orders of magnitude was increased. When mixtures comprised multiple candidate alleles, a proportion of LR's were reduced by multiple orders of magnitude. Candidate alleles having the greatest impact were those with frequencies ranging about 1%–7% in one of the FBI African American/Bahamian/Jamaican, Caucasian, and Southwest Hispanic populations and zero observations in another population. Due to the large expected frequencies of candidate alleles and their impact on LR's, it may be necessary to report statistics for considering whether mixtures comprise contributors of different populations.

### Probabilistic Genotyping, STRmix™, Mixture Interpretation

## B38 A Two-Trace Problem in Probabilistic Genotyping: Should the Evidence Be Combined or Not?

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**Learning Overview:** After attending this presentation, attendees will develop an understanding of the trade-offs between reporting comparisons of a person of interest to multiple evidential profiles separately or jointly.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness about the benefits and pitfalls of combining DNA evidence.

Casework laboratories routinely perform comparisons of persons of interests to DNA profiles obtained from crime scenes using probabilistic genotyping. When two evidential profiles are obtained from a single crime scene, it is standing practice in most laboratories to report a likelihood ratio statistic for each evidential sample measuring the degree of support for the person of interest contributing DNA to that sample. Although each of the two statistics can be used to evaluate support for contribution to one sample at a time, the two statistics do not necessarily give enough information to evaluate whether or not the Person Of Interest (POI) contributed to both samples. In the simplest case, we can consider two evidence samples that yield a complete profile that match each other and a person of interest. Considering this evidence jointly has been referred to as the two-trace problem, and evaluation in this framework has previously been explored.<sup>1,2</sup> A more complex extension is to consider partial or mixed DNA profiles, and the potential for a POI to have donated to a component of neither, one, or both profiles. These propositions can be investigated using a statistic that considers the two evidential profiles jointly.<sup>3</sup> Depending on case circumstances, the joint-contribution proposition may or may not be a more relevant proposition for a finder of fact. This two-trace problem is explored in this presentation using an example illustrating the trade-offs involved in deciding between reporting separate statistics, a joint statistic, or both.

Assume that two traces are obtained from a crime scene and that it is not certain whether or not these two traces originate from the same source. Both traces are only weakly informative of the genotype of the donor(s) because of low quantity or quality of the DNA. A suspect is apprehended and a reference profile is compared to each of the two traces. Because the evidential traces are of low quality, the two likelihood ratios are only weakly informative. Although it is possible to compute a statistic for joint contribution to both samples, the suspect does not have to concede in court that the two traces are of common origin. Moreover, it may be anti-conservative toward a defendant to wrongly assume a common origin. This suggests that presenting statistics combining evidence in court can be problematic. On the other hand, it is shown that if a common origin is correctly assumed, the joint statistic is more powerful, meaning that unrelated persons can be excluded more strongly. Specifically, it is shown that it is possible for the suspect to be categorically excluded as a donor to both samples while the two separate statistics are inclusionary. This means that it is not conservative either to only present the individual statistics, nor would it be conservative to present a minimum of the individual statistics. In conclusion, it depends on case circumstances as to whether or not the evidence should be combined.

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### Combining Evidence, Probabilistic Genotyping, DNA Mixtures

## B39 The Generation and Comparison of Various Short Tandem Repeat (STR) Stutter Positions and Longest Uninterrupted Stretch (LUS) Stutter Settings for a Probabilistic Genotyping Software Following Various Electrophoretic Protocols

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**Learning Overview:** After attending this presentation, attendees will be more familiar with the capability of the MaSTR™ software by SoftGenetics, LLC, to incorporate traditional and non-traditional stutter parameters for probabilistic genotyping and how appropriate stutter filters can affect likelihood ratio calculations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the increased capability of the MaSTR™ software to perform probabilistic genotyping on complex DNA mixtures. This presentation will illustrate the importance of using LUS as well as non-traditional stutter positions in the interpretation of forensic STR profiles containing contributions from multiple donors.

Stutter is a common DNA interpretation artifact that occurs as a result of strand slippage of polymerase during the elongation phase of Polymerase Chain Reaction (PCR). The two most commonly observed stutter artifacts are back stutter and forward stutter. Back stutter is well defined in literature, which allows filters to be applied during Short Tandem Repeat (STR) analysis. Forward stutter peaks are less common and occur at a lower frequency than backward stutter peaks.<sup>1,2</sup> Some of the features that affect the amount of stutter observed include Longest Uninterrupted Stretch (LUS), STR locus, total number of base pairs within the repeat, and the total number of repeats of the allele.<sup>3</sup> Studies have shown that LUS is a better predictor of expected stutter percentage compared to the total number of repeats in an allele.<sup>2</sup> Research has also shown that a longer LUS often leads to increased stutter and reduced amplification (lower peak height).<sup>2,3</sup> The ratio of the stutter peak height to that of the main allele peak is used to calculate the percentage stutter observed at a particular locus and can sometimes be used to differentiate stutter peaks from true allelic peaks of minor contributors in a mixed DNA.<sup>5</sup> DNA samples submitted to forensic labs for analysis are often composed of more than one contributor and each contributor may make up varying proportions of the sample. This makes analysis difficult and requires the use of statistical analysis and interpretation methods that are proficiently applied in continuous models of probabilistic genotyping methods.

Continuous probabilistic genotyping methods involve the use of weighted genotypes in the calculation of likelihood ratios. This requires the use of extensive information from the evidence sample such as variability of peak heights in heterozygote and homozygote alleles, stutter ratio and percentages, and probability of allele drop in and drop out.<sup>3-5</sup> MaSTR™ software is a fully continuous probabilistic genotyping software that uses Markov Chain Monte Carlo (MCMC) algorithms to generate weighted genotypes that are used in the calculation of likelihood ratios. The MaSTR™ software has been validated for use in forensic labs, using Scientific Working Group on DNA Analysis Methods (SWGAM) and Organization of Scientific Area Committees (OSAC) guidelines, and it has been shown to give accurate results when tested on sample mixtures with two to five DNA contributors.<sup>5</sup>

This study focuses on identifying locus-specific, non-traditional stutter products, developing new stutter filters based on these non-traditional stutter positions, and applying these filters to the new version of the MaSTR™ software. Large data sets (30+ single source profiles) from multiple PCR amplification kits were collected and analyzed to locate traditional and non-traditional stutter products. Appropriate allelic stutter filters for non-traditional stutter locations including LUS stutter amplicon products were calculated. Once the stutter percentages were calculated, they were imported into MaSTR™ software. With the release of a new version of MaSTR™ this fall, the new calculated stutter positions will be included. A performance check will be performed following SWGDAM guidelines. The performance check will utilize a set of mixtures known to be affected by stutter, and the genotype weight effects of the new stutter filters will be evaluated. Preliminary examination of the datasets indicates the prevalence of n-2 and n-8 non-traditional stutter products, with n-2 stutter being prevalent in marker D1S156 and n-8 stutter positions being prevalent in a majority of targeted markers. The percentage of the non-traditional stutter positions in the different STR markers will be calculated once the datasets have been examined in detail. The new stutter filters generated from this project and applied to the new version of the MaSTR™ software can be adopted by forensic labs for interpretation of mixed STR profiles.

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### Stutter, Probabilistic Genotyping, Forensic Science

## B40 The Generation of a Universal Protocol Data Set to Validate Probabilistic Genotyping Software for Uniformity Between Laboratories

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**Learning Overview:** After attending this presentation, attendees will understand: (1) if a shared data set can be used by laboratories for the validation and verification of probabilistic genotyping calculations on mixed DNA profiles using MaSTR™ software, and (2) how to implement suggested parameters and guidelines for MaSTR™ software into their workflow for mixture interpretation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guidelines to laboratories with limited resources and by introducing a sustainable avenue for switching from limited binary approaches to the more statistically powerful approach of probabilistic genotyping using the software MaSTR™ software.

Forensic scientists have been utilizing DNA analysis as an investigative tool for over 30 years. Samples collected from crime scenes often contain multiple contributors, degraded DNA, and/or low template DNA, which confound the interpretation process.<sup>1</sup> These complex mixtures are also often characterized by an increased presence of stochastic factors that further complicate interpretation.<sup>2</sup> Crime labs in the United States have historically used a binary method to interpret and perform statistical analysis on mixtures. As the mixtures produced from crime scene samples have become more complex, however, many labs have started to switch over to the non-binary approach of probabilistic genotyping, which eliminates the need for binary thresholds and increases the statistical power of interpretations.<sup>3</sup> A fully continuous probabilistic model uses peak heights and a variety of parameters to identify which contributor genotypes are best described by the data and then calculates a Likelihood Ratio (LR) to provide a statistical weight for the hypotheses chosen for court reports.<sup>4</sup>

Despite the obvious advantages to the probabilistic approach, the switch to the probabilistic approach has been slow in the United States.<sup>2</sup> Traditionally, laboratories have conducted an end-user validation to ensure that the software works as intended in their lab, using their protocols and instrumentation to produce data sets for this evaluation.<sup>5,6</sup> This process can take a substantial amount of time and resources away from casework. Many laboratories, therefore, continue to analyze complex mixtures using less informative, traditional approaches or report these mixtures as uninterpretable or inconclusive. This work examined the feasibility of generating a universal data set that could be utilized by laboratories interested in validating MaSTR™ probabilistic genotyping software in their laboratories, alongside a smaller data set created in-house for verification. In this study, software parameters (e.g., variance factor for peak height ratios) were validated using a large data sets of 44, 30, 20, and 10 single-source GlobalFiler™ profiles run through the MaSTR™ software in various replicates, depending on data set. From these large data sets, subsets of Short Tandem Repeat (STR) profiles were used to create multiple smaller data sets of varying sizes from which software parameters were determined. Variation within parameters between the large and small data sets were then analyzed to evaluate the extent of the variability between running data sets of varying sizes. This process was then repeated to determine the variation between data sets generated using different amplification kits. Data sets will be generated from the same amplification kits under different conditions, (e.g., different labs, PCR cycles, Capillary Electrophoresis [CE] injection times, etc.). The software parameters between these data sets will then be compared to determine if the variability between the parameters is significant. Finally, MaSTR™ software will be used to calculate LRs for the same DNA mixtures, using all of the data set parameters, and the variability between the calculated LRs will be assessed.

Preliminary results showed that the variability in generated parameters, between data sets of varying sizes was minimal. These preliminary results indicate that the variation within parameters can be effectively modeled for the MaSTR™ software, using all of the data set parameters generated from data sets of at least ten STR profiles. Some of the data set parameters generated from data sets of four STR profiles exhibited less variation than the larger data sets. These results indicate that for data sets with four or less profiles, the characteristics of the individual profiles impact the extent of variation within parameters. This study aims to provide a more sustainable alternative to a full in-house validation, which requires the use of unique internally generated data sets for the validation of MaSTR™ probabilistic genotyping software.

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### Probabilistic Genotyping, STR Analysis, Software Validation



## B41 How to Deal With Low Likelihood Ratios (LRs) in Mixed DNA Samples

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**Learning Overview:** After attending this presentation, attendees will better understand a method of how to deal with a low LR when making inclusionary statements about a Person Of Interest (POI) in a mixed DNA sample.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a graphical tool for the comparison of low LRs produced for minor contributors in mixed DNA samples to the general population. This can be used by the expert as an additional check for an exclusion opportunity and as a communication tool for helping the fact finder understand how the POI compares to the population at large.

The reporting of an LR calculated from probabilistic genotyping software has become more popular since 2015 and has allowed for the use of more complex mixtures at court. The meaning of “inconclusive” LRs and how to communicate the significance of low LRs at court is now important. Typically, mixtures that result in LRs <1,000 for a POI are those that are made up of multiple contributors and/or where the contributor position that best fits the POI has relatively high levels of drop-out. These mixtures are a challenge to interpret, but doing so is the only way to make exclusionary interpretations of the POI. However, when the human expert cannot exclude, or said another way, makes an interpretation that the POI is included as a possible contributor, the next step is to calculate the LR. When the resulting LR is small, but greater than one, it can be a challenge for the expert to communicate the meaning of a low LR to the jury.

Some have advocated the use of a verbal scale as an attempt to help communicate the meaning of low LRs to the fact finder.<sup>1,2</sup> This may be quite useful at times, although the verbal scale is related to the LR in a general manner and is not specific to the sample in question. It may be more helpful to have a communication tool that is directly related to the deconvolution and specific genotypes of the sample in question.

Gill and Haned proposed using a non-donor performance test as a measure of how robust the LR is for POI compared to a distribution of LRs from a population.<sup>3</sup> Understanding the use of a non-donor distribution may enhance communication between scientists and the court when discussing the significance of a low, but greater than 1, LR for low-level contributors in mixed DNA samples. The use of the database comparison function in the probabilistic genotyping software STRmix™ allows for this check to be performed on a routine basis.

A tool is presented here that uses the distribution of sub-source (DNA profile) LRs obtained from non-donors as a method for assisting in communicating the value of the sub-source LR for a POI.<sup>4</sup> The non-donor distribution is useful for examining calibration and discrimination for profiles that have produced LRs less than approximately 1,000. The output of the tool is a graph that can show the position of the LR for the person of interest set against the non-donor LR distribution. An LR for a POI that is less than 99.9% of the non-donor LRs would be considered uninformative.

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### Likelihood Ratio, DNA Mixtures, DNA

## B42 Microhaplotypes for Kinship Analyses

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**Learning Overview:** After attending this presentation, attendees will understand the potential application of Microhaplotype (MH) biomarkers in kinship analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing MHs as a supplemental tool to conventional Short Tandem Repeat (STR) typing analysis for kinship inference.

MHs are emerging biomarkers of at least two Single Nucleotide Polymorphisms (SNPs) associated in multiple allelic combinations within 300bp. The multi-allelic nature of MHs make them more informative than a Single Nucleotide Polymorphism (SNP) locus and useful for forensically relevant applications, including mixture deconvolution and ancestry inference on massively parallel sequencing platforms. In addition, they may prove helpful in kinship inference due to their low mutation rate. However, no study has fully demonstrated the potential of these markers in kinship inference. This study aimed to fill this gap by testing the utility of MHs in paternity and kinship testing by testing different scenarios.

An initial set of 347 individuals from four United States population groups (88 Afro-American [AA], 114 European-American [EA], 102 Southwest Hispanic [His], and 43 East-Asian American [EAA]) was genotyped using a 74 MH bioassay on the Ion S5™ System sequencing platform.

Allele frequencies for each population were calculated. A total of 1,000 simulation tests were performed for each population to determine Likelihood Ratio (LR) thresholds under the following four kinship scenarios: parent-child, full-siblings, half-siblings, and cousins. To achieve this, the commonly used and forensically relevant Familias software v. 3.2.8 was tested to take MH data.

Overall, the LR distribution median values ranged from  $10^{14}$  to  $10^{12}$  for parent-child pairs, from  $10^{10}$  to  $10^{11}$  for full-siblings pairs, and from  $10^2$  to  $10^3$  for half-sibling pairs. The distribution of LR median values for cousin's was found to be approximately equal to one, thus suggesting the need to use a larger marker assay to better infer this kinship scenario.

Subsequently, a total of 1,000 simulation tests were performed on 29 autosomal STRs using allele frequencies from the National Institute of Standards and Technology (NIST) dataset. The distribution of LR median values for parent-child and full-siblings was found to be approximately equal to  $10^9$  and  $10^7$ , respectively, while for half-siblings and cousins, both were approximately equal to one.

Overall, the high LR values obtained using the 74-MH bioassay, in comparison with LR values achieved with commonly used autosomal STR markers, supports the effectiveness of these new loci in kinship inference. In addition, the ability to distinguish close (parent-child and full-siblings) and more distant (half-siblings) familial scenarios highlights the possibility of solving complex family pedigrees, which deserves further investigation.

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### Microhaplotypes, Kinship Inference, Likelihood Ratios



## **B43 Mixture Software Is Invalid for Touch DNA**

*Charles H. Brenner, PhD\*, DNA-VIEW, Oakland, CA 94611-1336*

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**WITHDRAWN**

## B44 The Study of Using Next Generation Sequencing (NGS) Technologies to Analyze Mixed DNA Patterns

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**Learning Overview:** The goal of this presentation is to analyze the DNA mixture results obtained through capillary electrophoresis and NGS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the DNA mixture results using NGS.

In forensic science laboratories, capillary electrophoresis is currently employed to perform routine analysis of Short Tandem Repeat (STR) fragments and DNA sequences. However, the technique has limited discrimination for mixed forensic evidences. The DNA quantity of most forensic evidence is extremely small, and the mixing ratio is unknown. Therefore, it is difficult to identify trace components of DNA in mixed samples by capillary electrophoresis. NGS can overcome the problem of excessively large proportions of DNA in mixed samples by increasing the sequencing depth, and can analyze Single Nucleotide Polymorphism (SNP) or mitochondrial DNA sequence to assist in the study of DNA composition in mixed forensic evidence.

In this study, a total of 28 forensic cases were collected and analyzed through both capillary electrophoresis and NGS, including human STR, human mitochondrial HV1 and HV2 sequence, and animal mitochondrial 12S rRNA, 16S rRNA, and Cyt b sequences. A comparison of the results obtained through two methods was performed to validate the accuracy and reliability of NGS technologies. Moreover, NGS technologies can further aid to identify the sources of two-person mixed samples. Among the 15 DNA mixed samples analyzed were STR and Y-chromosomal Short Tandem Repeat (Y-STR) DNA patterns using the above two methods, and four mixed samples were examined of human mtDNA HV1 and HV2 sequence. In addition, nine animal cases were analyzed, which included mtDNA 12S rRNA, 16S rRNA, and Cyt b sequences.

The results of this research are respectively stated as follows. Human STR: the 15 cases were analyzed by the above two methods. The 15 cases can be correctly detected by capillary electrophoresis; however, 13 cases can be correctly detected by NGS. The detection rate of NGS was lower than traditional capillary electrophoresis due to the low DNA quantity of two cases. The detection rate of STR DNA of the remaining 13 cases of NGS was higher than traditional capillary electrophoresis. The study found that increasing the sequencing depth or supplementing with SNP sites can assist in the judgment of mixed samples. Human mtDNA: NGS analysis of forensic mixed evidences of mitochondrial DNA can break through the dilemma that capillary electrophoresis can only study the existence of two kinds of bases in mixed samples, and it is not easy to quantify the ratio. Animal species identification: Capillary electrophoresis only detected five cases, and the remaining four cases were not detected. The NGS technologies are more sensitive than capillary electrophoresis. Except for one case that was not detected due to severe decomposition, the other eight cases were all detected mitochondrial DNA sequences.

In conclusion, the NGS method still needs to invest a lot of manpower, material resources, and time, combined with molecular biology, forensic sciences, and statistical analysis to effectively use this huge amount of information to help identify forensic evidences. It is hoped that in the future we can continue to refine NGS technologies and achieve a forensic energy that could not be achieved in the past.

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### NGS, DNA Mixtures, DNA Analysis

## B45 A Performance Evaluation of the VeriFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit for Single Cell Forensics

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**Learning Overview:** The goal of this presentation is to inform attendees about how a standard, commercial Short Tandem Repeat (STR) profiling kit performs when utilized to genotype single cells and how single cells can be used to resolve mixed samples in forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that single cell analysis can be used to resolve complex mixed samples using regular commercial STR profiling kits. The analysis goes deep into the performance of each locus, evidencing behaviors that are not visible when working on bulk DNA.

This presentation will show that single cells are a unique invaluable resource of clean and unique profiles of each contributor in a mixed sample and that isolation and profiling of pure single cells can lead to the solution of cases with multiple contributors. For single cell forensics to be practically usable, it's necessary to have an appropriate digital cell-sorting device and high-performance commercial kits for STR profiling. This work evaluated the performance of the VeriFiler Plus™ PCR amplification kit with 25 multiplexed markers. The analysis of performance goes deep into each locus, evidencing behaviors that are not visible when working on bulk DNA, and it is finally demonstrated how forensic single cell analysis enables statistical confirmation of true loci vs. drop in peaks by double cell allele calling.

Mixed samples are one of the last unresolved challenges in forensic genetics. Solutions to overcome this problem have been adopted or proposed both on the sample preparation and on the data analysis side. Yet none has reached the sensitivity and non-ambiguity required for expert witness reporting in court. Furthermore, complex mixtures where two or more contributors share the same type of body fluid (sperm, blood, or epithelial) represent a higher level of complication since there is no available technique to phenotypically distinguish nor isolate cells of the same type from different individuals.<sup>1</sup> Single cell forensics enables us to resolve the problem of complex mixtures, allowing us to deduce each contributor's profile through a collection of single cells representing each, in purity, a single contributor to the mixed sample. Existing STR PCR amplification kits are not officially validated for DNA input below 0.5ng and, although most of them have the capability to produce detectable amplicons from single cells (~3–6pg), a performance evaluation is needed in order to adopt optimized protocols when attempting single cell analysis.

A simulated mixed forensic evidence, containing White Blood Cells (WBCs) and Sperm Cells (SCs) was generated in the laboratory. Cells from the dried swab were resuspended, fixed, and stained according to the DEPArray™ Forensic SamplePrep kit protocol and isolated on a DEPArray™ NxT digital sorter. A total of 30 single WBCs and 14 SCs were recovered, individually lysed, and PCR amplified using 29, 30, and 32 cycles with the VeriFiler™ Plus™ kit using half-volume reaction (12.5µl). Capillary Electrophoresis (CE) separation was performed for all samples on an ABI® 3500 Genetic Analyzer and GeneMapper® ID-X (v1.5) was used for data analysis.

PCR performance was evaluated in terms of Completeness and Concordance for the three cycling conditions in order to identify the optimal one. Overall, results show that with increasing PCR cycle numbers, the allele detection rate grows and the system reaches levels of completeness higher than 80% at both 30 and 32 cycles on both WBC and SC, in agreement with previous findings on different kits.<sup>2,3</sup> The appearance of a drop in peaks and unconventional height of stutter bands are the most common artifacts that could impact single cell STR profiling accuracy.<sup>3,4</sup> In this evaluation, at 29 cycles no detectable unspecific peaks were noticed, producing clean profiles with 100% concordance. One additional cycle introduced a minimal number of drop in peaks (1%) and a few more unconventional stutter bands (4.1%). Reaching 32 cycles, a more-than-proportional increase in both drop-ins and stutters was observed. Considering these trends, 30 cycles seems to be the best compromise to maximize the number of informative alleles while still maintaining a very low level of non-specific peaks. Utilizing Double Cell Allele Calling to confirm real positive, only those found in at least two cells, it was found that with just six cells belonging to the same contributor, it's possible to match maximum completeness with very high concordance.

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### Mixed Samples, Single Cell, Digital Sorting

## B46 lusSTR®: An Open-Source Python® Package for Translating Forensic Short Tandem Repeat (STR) Sequences Into Interpretable Formats

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**Learning Overview:** After attending this presentation, attendees will understand the capabilities of lusSTR®, a new software program for translating human DNA sequences of STR loci into more user-friendly representations.

**Impact on the Forensic Science Community:** lusSTR® implements an automated method for converting human DNA sequences of STR loci derived from Next Generation Sequencing (NGS) into more intelligible annotation. Laboratories can utilize the lusSTR® program to assist validation of STR sequencing protocols, to facilitate interpretation of STR sequence data, and to more easily compare STR sequences produced by different NGS assays and analysis packages.

While recent advances in human forensic DNA technology have provided significant improvement in identification capabilities, they have also created additional challenges in analyzing and interpreting the resulting data. The ability of DNA sequencing to detect nucleotide-level variation at conventional forensic STR loci has revealed further individualization capabilities beyond the traditional length-based allele designation. However, the long sequence strings that result from NGS typing of STR loci are difficult for analysts to interpret in a consistent manner.

Currently, there is no standard for translating STR sequences into more human-readable forms, nor is there community consensus regarding what those forms should be or the portions of the amplified STR region they should include (e.g., repeat region only or including flanking sequence). In addition, depending on the NGS assay and analysis program employed, sequences for some loci may be reported according to previously acceptable reverse strand (3' to 5') alignment rather than the GRCh38-consistent forward strand reporting recommended by an International Society for Forensic Genetics (ISFG) DNA commission.<sup>1</sup> As a result, forensic laboratories investigating and validating NGS technologies for sequencing the STR loci face challenges in analyzing the sequence data produced in their internal studies, as well as difficulties comparing results across NGS assays, analysis programs, and STR datasets. The use of reference tables for sequence translation, while straightforward, requires that an exact match to the sequence be present in the reference set. Sequences not present in the table may require manual handling, which can produce inconsistent and error-prone annotation of these complex strings. An automated and flexible method for sequence string translation ensures consistent annotation and facilitates analysis of STR sequences both internal to a lab and across laboratories and datasets.

The open-source software program lusSTR® was developed to convert STR sequence strings to several different representations to allow for easier and more reliable comparisons between sequences. lusSTR® accommodates the autosomal, Y- and X-chromosome STR sequences produced using two commercial NGS assays: the ForenSeq™ Signature Prep kit and PowerSeq™ 46GY and uses as input either: a standard report produced from one of two STR alignment packages (ForenSeq™ UAS and STRait Razor v3) or a simple .csv file of sequences.<sup>2</sup> lusSTR® partitions sequences into core and flanking regions, reverse complementing as necessary, and produces an output file containing multiple locus-specific annotations per sequence.

This presentation will describe the lusSTR® program, detail the development and testing of lusSTR®, and depict how lusSTR® functions and may be used in the forensic setting. The multiple annotation types produced by lusSTR®, derived by an automated method, will provide analysts a more straightforward and reliable way to evaluate and compare forensic STR sequences.

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### Next Generation Sequencing, Short Tandem Repeats, Bioinformatics

## B47 A Routine Rape Case That Became Tricky (and Educational)

Mark Benecke, PhD\*, *International Forensic, Cologne, NRW 50520, GERMANY*

**Learning Overview:** After attending this presentation, attendees will understand why it is important to listen to the victim and relatives, who do not fully understand the objective DNA evidence, but who point in a relevant direction relating to procedural failures of police and crime labs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees learn how to understand claims of a victim's family who seem to simply not understand the science in a "simple" rape case but in fact detect major flaws in the procedure.

In a technically "simple" routine case of rape (anal and vaginal intercourse over one hour; bleeding from anal lacerations; in home of victim; probably no ejaculation; male person not an occupant and not a relative), the case never went to court due to the absence of male DNA and the "impossibility to find out if sexual intercourse had taken place." On the swabs, saliva from the victim but none of the possible offender was reported to be found by the federal state lab.

The woman and her family contacted a forensic research and consulting firm primarily because they wanted to know if no male DNA was to be expected after prolonged intercourse even without ejaculation and because, using the crime lab's DNA results, the family had calculated that "25%" of the DNA profile of victim and possible offender matched in clean controls (oral swabs). They claimed that the swabs had been mislabeled at the hospital/by the police and half of the samples (originally ten samples: four vaginal, two anal, two oral, and two possible offender) were reported missing.

Since the possible sexual incident was recorded on a mobile phone (sound only), which was considered to be relevant, the case was re-examined and it was found that not only different systems for DNA typing had been used (this explained the "25%" match between male and female DNA contributions) but also the labeling of the swabs had initially indeed been wrong. After inspection of the woman's underwear (different crime light sources; swabs), which had never been looked at before even though the possible rape was immediately reported to the police, DNA of the possible offender was found (Short Tandem Repeat [STR], Y-chromosome [Y]) on four locations of the pantyhose that had been worn by the woman before and after the intercourse and stored afterward without alterations. Since it is known that DNA may be transferred accidentally, especially at the waistband area, the original laboratory reports were checked for amounts and levels of DNA and to reconstruct the course of events on the relevant night.<sup>1</sup>

This presentation shows how procedural problems may arise even in a highly standardized, controlled, and certified federal state lab if routine procedures are applied too firmly. The rape case and its stains had to be taken out of the routine line, new samples had to be taken by an external forensic biologist from evidence not looked at before, and the statements of the relatives had to be checked in the files as well as experimentally.

An important lesson was to listen to clients even if their statements initially seem to be incoherent.

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### Rape, DNA, Expert Witness

## B48 Increasing the Speed and Efficiency of 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 understand how a microwave can be used to rapidly extract DNA for improving Rapid DNA analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how a microwave can be used to increase the speed and efficiency of extractions for rapid direct Polymerase Chain Reaction (PCR) analysis.

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 fieldable 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 less than 90 minutes and a newly developed ultra-rapid DNA system can yield genotypes in under 15 minutes.<sup>2,3</sup>

The problem is that the processes these instruments use to isolate DNA are slow and inefficient and may not be fast enough nor effective enough for certain sample types and critical time-sensitive intelligence applications.<sup>4</sup> Additionally, results from studies involving crime scene samples indicate the need to mitigate issues with sensitivity and PCR inhibition as well as improving the speed of rapid DNA systems. Processing difficult samples in remote locations is important for rapid on-site intelligence applications. Thus, there is a need to develop a more efficient front end for processing difficult samples in remote locations.

In this study, microwave DNA extraction using both conventional and computer-driven microwaves have been tested for rapid direct PCR. Previous research results have demonstrated successful microwave extraction from both eukaryotes and prokaryotes, including human bodily fluids and tissues.<sup>5-20</sup> Single-source human saliva, blood, and semen samples were prepared on replicate swabs at different total cellular amounts between 25–500 cells. Protocols using different microwave energy levels at no microwave energy, 300, 400, 500, 600, and 700W of energy at 40 seconds were tested first on replicate saliva swabs containing varying cellular loads. Improved DNA yield for samples microwaved at 300W for 40 seconds was observed. Further experiments with no microwave controls, and 100, 150, 200, and 300W microwave treatments at 40 seconds were carried out. Improved yields were again observed at 300W; however, some loss and variation was observed across replicates due at least in part to saliva sample heterogeneity. Short Tandem Repeat (STR) RapidHIT™ testing of replicate 1% saliva dilutions demonstrated increased allele detection and peak heights for the microwave-treated saliva samples on the Rapid HIT™ system. On average, microwaved replicates of 1% saliva swabs resulted in correct allele detection of 92% (34 of 37), whereas unmicrowaved replicates resulted in only 78% correct allele detection (29 of 37). Microwaved sample peak heights increased an average of 215 Relative Fluorescence Units (RFU) versus non-microwaved samples (with a range of 31–594 RFU for 26 alleles). For seven alleles, microwaved peak heights decreased an average of -198 RFU (with a range of -24 to -341 RFU).

All alleles were correctly detected at 10% saliva for both microwaved and non-microwaved samples.

In addition to testing the commercial RapidHIT™ systems, a rapid direct STR multiplex has been developed. The multiplex consists of the following loci: D10S1248, FGA, D8S1179, D7S820, AMELOGENIN, D2S441, D18S51, D2S1338, D21S11, TH01, and vWA. Results of optimization experiments under different combinations of primer concentration, cycling temperature, cycle number, and template has resulted in successful rapid 15 minute co-amplification of nine STRs with no extraction step other than microwave processing. Microwaved 1% saliva samples were directly amplified with no additional extraction resulting in increased peak heights over non-microwaved samples.

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, resulting in improved recovery of low template samples for rapid DNA analysis. These positive effects should greatly improve overall speed and success of rapid DNA processing for both laboratory and commercial systems.

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## Microwaves, DNA, Rapid

## **B49 Toward Developing a Forensically Relevant Single-Cell Pipeline by Incorporating Direct-to-Polymerase Chain Reaction (PCR) Extraction: Effects on Signal Quality and Allele Dropout**

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**Learning Overview:** After attending this presentation, attendees will better understand how direct-to-PCR extraction chemistry affects the DNA Electropherogram (EPG) signal garnered from single cells.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that four commonly used metrics of single-cell EPG's are significantly impacted by direct-to-PCR treatment employed. Along with demonstrating the impact of extraction treatment, this presentation will show the potential associated with single-cell pipelines for solving the complex DNA mixture problem.

The interpretation of mixture samples using a traditional pipeline, where the DNA of many cells are extracted together, is difficult since an unknown number of contributors of unknown concentration renders EPGs so complex that interpretation requires significant computational power to complete. An alternate to the bulk-processing pipeline is a single-cell one, where the sample is collected, and each cell is separated. The DNA is then extracted using, preferably, a direct-to-PCR treatment that is efficient and compatible with all forensically relevant downstream processes. Though single-cell pipelines have the potential to fill the gaps left by the bulk-processing pipelines, it is a necessity to confirm novel extraction and interpretation strategies meet forensic requirements.

In this study, the feasibility of implementing single-cell pipelines into the forensic process by exploring whether allele dropout rates are cell-dependent is demonstrated. Four metrics of EPG signal quality (i.e., allele detection rates, peak heights, peak height ratios, and peak height balance across low to high molecular weight Short Tandem Repeat (STR) markers) obtained with four direct-to-PCR extraction treatments: forensicGEM<sup>®</sup> Saliva; DEPAArray<sup>™</sup> LysePrep Kit; Direct PCR Lysis Reagent; and Arcturus<sup>®</sup> PicoPure<sup>™</sup> DNA Extraction Kit were assessed. Each of the four methods was used to extract DNA from 102 single buccal cells, whereupon the amplification reagents were immediately added to the tube and the DNA was amplified/injected using post-PCR conditions (laboratory conditions: GlobalFiler<sup>™</sup> PCR Amplification Kit, 30 PCR cycles, and 25-second injection on ABI<sup>®</sup> 3500 Genetic Analyzer) known to elicit a Limit of Detection (LoD) of one DNA molecule. The results show that 77% of cells (313 out of 408 single buccal cells), regardless of extraction treatment, rendered EPGs with at least a 50% true positive allele detection rate, and that allele drop-out was not cell-independent. Permutation tests demonstrated that extraction treatments significantly impacted all metrics of EPG quality. Notably, the Arcturus<sup>®</sup> PicoPure<sup>™</sup> extraction method resulted in the lowest median allele drop-out rate, the highest median average peak height, the highest median average peak height ratio, and lowest median values of EPG sloping. It is, therefore, concluded that implementing single-cell pipelines into casework is feasible and demonstrated that inferential systems assuming cell independence is not ideal for the probabilistic interpretation of a collection of single-cell EPGs.

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**Forensic DNA, Single-Cell Forensic Analysis, Direct-to-PCR Extraction**

## B50 Analyzing the Effect of Cleaning Products on Presumptive Blood Testing Kits

Heather Kavaloski\*, Texas A&M University, Forensic Science, College Station, TX 77840; Adrienne Brundage, PhD, Texas A&M University, College Station, TX 77845

**Learning Overview:** The goal of this presentation is to provide a better understanding of the ways in which cleaning products may negatively affect presumptive blood tests.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by equipping crime scene professionals with an increased understanding of chemical interactions in the field. An increase in the knowledge of these chemical reactions between cleaning products and presumptive blood tests will reduce the concern of missed blood evidence and DNA at a crime scene. Establishing and maintaining the criteria for truth in evidence is crucial to forensic investigations. This presentation intends to improve the ability of crime scene professionals to perform well in uncertain conditions within a crime scene.

This presentation will increase the understanding of chemical interactions and presumptive blood tests performed in the field. An increase in this knowledge will not only improve performance, but will also reduce the concern of missed blood evidence and DNA at a crime scene. Past research has found that certain cleaning products negatively interfere with presumptive blood testing.<sup>1,2</sup> This research presentation will impact the forensic science community by adding to the current criteria for truth in evidence.

This research focused on the hypothesis that if consumer-grade cleaning products are applied to blood stains, then they will negatively interfere with presumptive blood testing results. Phenolphthalein (PT), Leucocrystal Violet (LC), and Tetramethylbenzidine (TB) presumptive testing kits were used to test blood on tiles cleaned with common products. Approximately 10mL of blood was left to dry for 20 minutes on seven tiles. The blood was cleaned using paper towels and 10mL of one of six common cleaning products: Method® glass cleaner; Dawn Essentials® dish soap; Wet Ones® disinfecting wipes; Citrasolve® cleaner and degreaser; HDX® citrus degreaser; and Mean Green® degreaser and cleaner. The control tile was wiped with a paper towel but not with a cleaning product. The tiles were then left for one week (seven days) to simulate the time between the commission of a violent crime and the crime scene investigation process. Each sample was then tested with the PT, LCV, TB and Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) presumptive blood tests.

The simple H<sub>2</sub>O<sub>2</sub> test did not yield any results. PT was not affected by any of the cleaning products while LC and TB were unable to detect blood on tiles cleaned with degreasing products. It can be concluded that the results obtained in this research study successfully identified false negatives on the LCV and TB presumptive blood testing kits. This research has shown that with the LCV presumptive blood test, Citrasolve® and Mean Green® both yielded false-negative results. The TB presumptive blood test yielded false negative results with Mean Green®. Chemical interactions between the presumptive tests and components of the cleaning products have been identified as the cause behind the false negative results.<sup>1,2</sup> Cationic compounds within the LCV reagent and the chelating agents in the degreasing cleaning products have been identified as the likely sources of interaction.

This research presentation will explain the characteristics of the chemical compounds that affected the presumptive blood testing kits. It will also illustrate the chemical reaction that occurs to interfere with the test in the field.

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### Presumptive Testing, Latent Blood Detection, Crime Scene

## B51 Recovery of DNA From Surfaces of Handguns: Targeting Relevant Sampling Areas

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**Learning Overview:** After attending this presentation, attendees will be familiar with collecting DNA samples from individual areas of handguns, a process that may help preserve evidence and minimize the generation of artificially mixed DNA profiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing practitioners as to which specific area(s) of a handgun may be best suited for the recovery of DNA that generates useful results for probabilistic genotyping.

Because many factors potentially influence the transfer of DNA onto a firearm, such as the frequency of handling by one or multiple individuals, the frequency of cleaning, and how and with what the weapon is cleaned, designing an experiment that accounts for all variables is nearly impossible. To provide guidance on sample collection from firearms to assist in criminal investigations, testing many complex, interrelated variables must include a comprehensive understanding of DNA transfer mechanisms.

This study was designed to systematically test whether there is a detectable difference in the quality and quantity of DNA detected on four specific areas of the firearm: grip, trigger, slide, and magazine. Eleven handguns owned by 11 different law enforcement personnel were used in this study. A questionnaire was completed by each participant that ascertained length of ownership, length of daily contact, frequency of cleaning, time since last fired, and accessibility by other individuals. All weapons were Glock<sup>®</sup> of varying calibers. Gun ownership ranged from less than a year to 25 years. Participants reported that service weapons were kept on their person 8+ hours per day and fired within the last 12-month period. Regular cleaning was reported to occur every two weeks, monthly, or bi-annually. Seven officers reported that no one else had access to their service weapon while four officers reported that a significant other had access.

Samples from the four designated areas of the firearm were collected separately using a single-swab technique. Each swab was moistened with 100µl of sterile Phosphate Buffered Saline (PBS). A total of 44 samples were extracted utilizing the QIAamp<sup>®</sup> DNA Mini Kit per manufacturer's instructions. Extracted DNA was amplified with the GlobalFiler<sup>™</sup> Polymerase Chain Reaction (PCR) Amplification Kit and analyzed on an AB SCIEX<sup>™</sup> 3130xl genetic analyzer. STRmix<sup>™</sup> v 2.6.1 was utilized to aid in interpretation. Deconvolution of any mixtures was completed before comparisons to known reference samples from the owners. The National Institute of Standards and Technology (NIST) 1036 database was used to calculate Likelihood Ratios (LRs). The Scientific Working Group on DNA Analysis Methods (SWGAM) scale of verbal qualifiers was used to express the degree of support for a specified proposition (H<sub>1</sub>—owner of gun is a contributor) relative to an alternative proposition (H<sub>2</sub>—unknown, unrelated individual is a contributor).<sup>1</sup>

Data was obtained from all 44 samples. The amount of DNA recovered varied from area to area and from gun to gun. On average, more DNA was obtained from the slides and magazines and less DNA was obtained from the triggers and grips. Single-source DNA profiles (9% grip, 27% trigger, 36% slide, 27% magazine) and mixed DNA profiles of two or three contributors (91% grip, 91% trigger, 64% slide, 73% magazine) were observed. There was no discernable pattern as to Number Of Contributors (NOC) based on area. Interestingly, mixtures of two to three contributors were obtained from weapons that were reported to be accessible only by the officer.

The LRs for all grip, slide, and magazine samples provided very strong support (i.e., LR ≥ 1,000,000) in favor of H<sub>1</sub>. Varying LRs were obtained from the trigger samples. For the majority of the trigger samples, the LRs provided very strong support in favor of H<sub>1</sub>. In one instance, the LR provided moderate support (i.e., 100 ≤ LR ≤ 9,999) in favor of H<sub>1</sub>. In this sample, data was only detected at 29% of the GlobalFiler<sup>™</sup> loci. Interestingly, the LR (i.e., 2 ≤ LR ≤ 99) for one trigger sample provided limited support in favor of H<sub>2</sub>. In this sample, data was detected at 54% of the GlobalFiler<sup>™</sup> loci.

This study has demonstrated that useful DNA profiles can be obtained by single swabbing certain parts of a handgun. Based on the results, the slide and magazine samples appear to provide the best results. Although the amount of DNA on average obtained from the grip samples was less, useful results were still obtained from this area of the weapon. Trigger samples appear to provide the least impressive results.

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### Transfer DNA, Handguns, Probabilistic Genotyping

## B52 STK<sup>®</sup> Sperm Tracker as a Presumptive Test for Semen Stain Detection

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**Learning Overview:** After attending this presentation, attendees will understand the results of a study designed to test the performance and sensitivity of STK<sup>®</sup>, an alternative method for semen stain detection, compared to traditional serological techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the benefits and challenges of STK<sup>®</sup> as a presumptive semen identification technique on sexual assault evidence items.

Traditional methods for semen stain identification, such as Alternative Light Sources (ALS) and the Acid Phosphatase Spot Test (AP), can present issues for forensic examiners, especially in regard to sensitivity, specificity, and efficiency. To address these challenges, STK<sup>®</sup> was developed as a rapid, specific, and non-toxic method for semen stain detection on sexual assault evidence. STK<sup>®</sup> is a presumptive test for human semen identification, reacts specifically with human prostatic acid phosphatase, and is available in paper and spray versions.

This study evaluated STK<sup>®</sup> paper and spray with various dilutions of semen stains on cotton fabrics, per the manufacturer's specifications, as well as with slight protocol modifications. Results showed that STK<sup>®</sup> paper performed best on blue denim, white denim, and a black bedsheet, while STK<sup>®</sup> spray performed best on a white bedsheet when following the manufacturer's protocol. Modifications, including the simultaneous application of STK<sup>®</sup> spray and paper as well as increasing STK<sup>®</sup> spray concentration, were made to determine if signal detection could be improved; however, those changes only helped in a limited number of instances.

Additionally, this study tested STK<sup>®</sup> sensitivity by detecting semen stains on non-laundered and laundered denim and white bedsheets fabrics in comparison to ALS followed by AP. For non-laundered fabrics, ALS and AP performed the best overall on the blue denim, while STK<sup>®</sup> spray performed the best on white bedsheets. For laundered fabrics, signals were clearly visualized with ALS on white bedsheets and less so on blue denim, while faint signals appeared with STK<sup>®</sup> spray on white bedsheets only; STK<sup>®</sup> paper did not produce any signals on these fabrics. Finally, it was determined that STK<sup>®</sup> does not affect GlobalFiler<sup>™</sup> and Yfiler<sup>™</sup> Polymerase Chain Reaction (PCR) amplification for downstream Short Tandem Repeat (STR) analysis.

In summary, STK<sup>®</sup> showed promise in certain scenarios but nevertheless, traditional ALS in conjunction with AP may remain the preferred approach for presumptive testing of semen on the types of cotton materials tested here. STK<sup>®</sup> paper worked better than spray on thicker and darker fabrics; however, its set-up was slightly more cumbersome than any other test in this study. STK<sup>®</sup> spray performed well on white bedsheets, but less so after being laundered, and its ease-of-use may also be suitable for non-fabric evidence items and large areas in the field. Regardless, STK<sup>®</sup> spray may prove useful for forensic examiners when testing white bedsheets because it could replace the ALS and AP steps, helping to increase the efficiency and accuracy of semen serological testing. Additional testing of other fabrics and substrates is warranted.

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### STK<sup>®</sup> Sperm Tracker, Semen Identification, Presumptive Testing

## B53 Practical Applications of a Wet-Vacuum DNA Collection System

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**Learning Overview:** After attending this presentation, attendees will understand how a wet-vacuum is used for DNA collection in a forensic context as well as the results of five studies designed to evaluate its performance and potential case use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a wet-vacuum system that could serve as an alternative DNA evidence collection method and its potential use at crime scenes and/or laboratories.

Crime laboratories routinely receive large, porous, and/or irregular evidence items, which are difficult to sample for DNA testing with simple swabbing, tapelifting, or cutting techniques. A DNA collection system which utilizes wet-vacuum technology, called the M-Vac<sup>®</sup>, was designed for in-lab or in-the-field sampling of such challenging materials. The wet-vacuum dispenses pressurized sterile solution onto a surface and simultaneously vacuums cellular material into a sample collection bottle. To collect and concentrate the cellular material, the sample is filtered through a 0.45 $\mu$ M Polyethersulfone (PES) membrane in a two-stage filter unit. The membrane filter is then cut from the unit and processed for DNA extraction.

The studies presented here explore practical forensic uses of the wet-vacuum system: (1) assessment of a five-foot versus 40-foot length hose/solution line for recovering diluted 1/100 blood on glass; (2) performance of wet-vacuum collection and DNA concentration when Bluestar<sup>®</sup> was applied onto bloodstained painted drywall and automotive carpeting; (3) evaluation of an alternative, yet similar, DNA extraction protocol against the wet-vacuum manufacturer's recommended protocol with the EZ1<sup>™</sup> DNA Investigator Kit; (4) comparison of a single- versus double-filtration method for DNA concentration; and (5) investigation of other filter membranes (i.e., 0.2 $\mu$ M nylon, 0.2 $\mu$ M cellulose nitrate, and 0.2 $\mu$ M surfactant-free cellulose acetate membranes, compared to the recommended 0.45 $\mu$ M PES membrane for recovery of cell-free and fragmented HL60 DNA). All samples were evaluated for nuclear DNA (nDNA) quantity and quality using the Quantifiler<sup>®</sup> Human Plus DNA Quantification Kit and total nDNA yields were assessed for each study.

Results indicated that total nDNA yields recovered from 1/100 blood on glass with the wet-vacuum were comparable, regardless of hose/solution line length. Yet the use of Bluestar<sup>®</sup> seemed to reduce DNA yields from blood spotted on automotive carpet, possibly because of increased application of Bluestar<sup>®</sup> due to the absorbent nature of the carpet, while DNA yields recovered from blood with and without Bluestar<sup>®</sup> applied onto painted drywall were similar. In addition, there were no significant differences in recovery yields using different extraction protocols or for single- versus double-filtration. Lastly, the 0.2 $\mu$ M cellulose nitrate filter membrane significantly captured more cell-free, unfragmented DNA compared to the other filter types; however, fragmented DNA was mostly present in the filtrate, rather than on the membrane surface, for all filter types.

The various studies demonstrated here highlight that the wet-vacuum system can be used as a DNA collection tool in several practical forensic applications. Results provided no evidence that hose length, extraction protocol, or the number of filtrations affected DNA recovery, negatively or positively. However, Bluestar<sup>®</sup> reduced DNA quantities when applied onto absorbent materials but not a smooth surface. The use of 0.2 $\mu$ M cellulose nitrate showed significant improvement over the recommended 0.45 $\mu$ M PES membrane for high molecular weight DNA, suggesting that this alternative filter membrane may be more suitable when cell-free DNA is suspected to be present. However, more research is warranted to improve the retention of low molecular weight, fragmented/degraded DNA.

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### M-Vac<sup>®</sup>, Wet-Vacuum, DNA Collection

## B54 Using Hybridization Capture to Obtain Mitochondrial Genomes From Forensically Relevant Canids: Assessing Sequence Variation for Species Identification

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**Learning Overview:** After attending this presentation, attendees will have learned the common methods to identify vertebrate species in non-human forensic casework and the limitations of those methods when trying to distinguish closely related species. Based on these results, attendees will be introduced to other regions of the mitochondrial genome that can offer species resolution for closely related canids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing attendees to a method that can be straightforwardly used to generate full mitochondrial genomes: hybridization capture baits designed for a closely related species that isolate target DNA fragments for next generation sequencing. This method works well with low template and highly degraded samples and could be applied to other species groups.

The majority of DNA casework processed by forensic laboratories focuses on human samples, but material from canids (i.e., dogs, wolves, coyotes) can also be encountered. For example, dogs can be the victim of a crime or link a potential suspect to a victim or crime scene via their hair and biological fluids. Undomesticated canids, such as wolves and coyotes, can also be the center of forensic investigations in the United States, given that some species are endangered, meaning that hunting them in certain jurisdictions is illegal. Given many wolf species are highly similar morphologically, identification in the field by wildlife officers is often not straightforward and can be further complicated when incomplete specimens are available. Thus, molecular-based approaches are often used for canid species identification.

While some established methods using mitochondrial DNA targets can discriminate between *Canis* species, they are either not compatible with highly degraded samples (e.g., Cytochrome C Oxidase I [COI] barcode region is ~650bp) or they cannot differentiate closely related subspecies (most recent common ancestor is ~20,000 years ago). Although some United States laboratories regularly perform veterinary/wildlife casework, including canid identifications, their validated methods and the reference genetic databases they use are not publicly available. Thus, this study aimed to assess the utility of alternative regions in the mitochondrial genome for discriminating among forensically relevant canid species. To achieve this, a commercially available hybridization capture panel designed for the domestic dog (*Canis lupus familiaris*) to enrich entire canid mitochondrial genomes from Abor Biosciences™ was utilized. Briefly, this panel consists of biotinylated RNA “baits” that are complementary to the dog’s mitochondrial genome, permitting isolation of the whole mitochondrial genome from even highly degraded samples for downstream next generation sequencing. Given the baits will anneal when the template sequence is ~80% identical, it was hypothesized that the assay for the domestic dog would permit the recovery of full mitochondrial genomes from closely related wolves and coyotes. This study used this panel to successfully sequence full mitochondrial genomes for 53 samples (total input quantities as low as ~10ng), representing four United States forensically relevant canid species (coyote, wolf, Mexican wolf, and dog). While the full mitochondrial genome permitted discrimination between species and subspecies, this study also identified four ~200bp fragments from ND1, ND5, COI, and CYTB genes that could resolve the canids sampled in this study. The utility of these regions should be more fully assessed in future studies prior to implementation into casework, using forensic-type samples representing canids from diverse geographic areas.

### Hybridization Capture, Mitochondrial Genomes, Wildlife Forensics

## B55 An Evaluation and Comparative Analysis of Rapid DNA Analysis and Direct Amplification

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**Learning Overview:** After attending this presentation, attendees will better understand the differences between Rapid DNA analysis and direct amplification for the swift development of investigative leads for law enforcement.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic laboratories with the knowledge to determine if Rapid DNA analysis and/or direct amplification may be suitable for the swift development of investigate leads for law enforcement through an evaluation of the performance, efficiency, and cost of both systems.

Traditional forensic DNA analysis involves multiple steps and can be time consuming. In certain situations, it may be beneficial to speed up this process and obtain an interpretable DNA profile within a few hours. Rapid DNA workflows are optimized to produce DNA profiles in a relatively short amount of time, albeit from high-level, single-source DNA samples, such as buccal swabs. The application of these rapid DNA workflows for the processing of casework samples, which do not always offer a high quality and/or quantity of DNA, is of great interest to the forensic community as well as law enforcement.

Commercially available rapid DNA instruments have been marketed toward law enforcement agencies for the analysis of casework samples to generate investigative leads quickly. To provide law enforcement with options for more rapid and efficient processing techniques that lead to real-time investigative leads, the Houston Forensic Science Center launched a pilot project to potentially determine the most suitable rapid DNA workflow to integrate into an accredited Forensic Biology Section and undergo a more significant validation. To aid in this determination, this study evaluated the performance, efficiency, and cost of a single Rapid DNA Analysis system and several direct amplification kits. Samples ( $n = 450$ ) of various types and concentrations, deposited on various substrates were processed using the Applied Biosystems® RapidHit™ ID system (RHID) utilizing INTEL cartridges and the following direct amplification kits: Applied Biosystems® GlobalFiler® Express PCR Amplification Kit lysed in Prep-n-Go™ Buffer, Promega® PowerPlex® Fusion 6C System lysed with Casework Direct System, QIAGEN® Investigator® 24plex QS and QIAGEN® Investigator® 26plex QS Kits lysed with Investigator® Casework GO! Kit.

The success rate of each rapid DNA system was measured by the percentage of complete and concordant genotypes generated at the 20 Combined DNA Index System (CODIS) core loci when compared to genotypes obtained from the traditional laboratory workflow. For GlobalFiler® Express, the success rate of obtaining a complete DNA profile was 95% for blood and 85% for semen. For PowerPlex® Fusion 6C, the success rate was 80% for blood and 95% for semen. For Investigator® 24plex QS, the success rate was 95% for blood and 95% for semen. For the Investigator® 26plex QS, the success rate was 100% for blood and 90% for semen. For RHID, the success rate was 100% for blood and 85% for semen. A failure rate of 3.3% due to instrument errors was observed with the RHID system. None of the systems produced full profiles with saliva or miscellaneous samples containing 1ng or less DNA. Concordance between rapid DNA systems was observed for 98.83% of the STR alleles compared. Five samples exhibited either a single drop-in event or multiple alleles, suggesting the presence of a contaminant.

The average analysis time for any of the direct amplification kits is approximately 3 to 3.5 hours regardless of the number of samples. The average analysis time for RHID analysis is approximately 1.5 hours per sample. The cost of the RHID system can range from \$220/sample down to \$90/sample. Considering only the cost of the chemistry and no overhead, supplemental reagents, or consumables, GlobalFiler™ Express is ~\$18/sample, PowerPlex® Fusion 6C is ~\$13/sample, Investigator® 24plex QS is ~\$23/sample, and Investigator® 26plex QS is ~\$22/sample.

Overall, both the RHID system and the direct amplification kits performed similarly. Both performed well with blood and semen samples, but variable results were obtained from saliva and miscellaneous samples containing  $\leq 1$ ng DNA. Both workflows have benefits and drawbacks that should be considered. While the RHID instrument can be mobile, the direct amplification workflow is restricted to the laboratory, but has the added benefit of processing multiple samples simultaneously. Reprocessing is also possible with both workflows. Both workflows coupled with an internal database can be powerful resources for developing timely investigative leads for law enforcement.

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### Direct Amplification, Rapid DNA Analysis, Investigative Leads



## B56 Trends of Trace DNA Profiles in Forensic Casework: A Broad Case Study

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**Learning Overview:** The goal of this presentation is to inform attendees about a case study of trace DNA samples across hundreds of forensic casework samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that this research seeks to better understand trace DNA as it pertains to actual forensic casework rather than experimental settings.

Detecting and analyzing trace (“touch”) DNA profiles has long been a challenge for forensic biologists. With the advent of more sensitive forensic Short Tandem Repeat (STR) amplification kits, a larger variety of sample types without identified bodily fluids can now be used as forensic evidence. However, analysis of trace samples often results in inadequate amounts of DNA or DNA that is degraded. Trace DNA samples can also result in complex mixtures that are difficult to interpret. Countless studies have been published by the forensic science community to better understand how trace DNA is deposited and transferred on a variety of surfaces. These past studies have typically focused on experimental conditions rather than on actual evidentiary samples collected from crime scenes.

This study’s purpose was to better understand the behavior of trace DNA evidence that has been observed in forensic casework. To achieve this, an in-depth study was conducted in which data was compiled and analyzed from over 600 trace DNA samples (more than 200 cases) from casework at the Utah Bureau of Forensic Services, comprising data that spans from 2014 to 2020. Metrics that were examined included evidence item type, small autosomal human DNA quantitation values, number of contributors in the sample (if that could be determined), and whether or not the sample yielded a comparable DNA profile. A comparable DNA profile obtained from the sample was ultimately what determined whether or not a sample “succeeded,” although this does not account for if the profile obtained was probative. The numerous types of evidence that were analyzed included ammunition, firearms, structures, vehicles, weapons, clothing, and a category of miscellaneous items. Clothing was also subcategorized based on whether the item was swabbed for the wearer’s DNA or for the profile of someone who had merely touched the garment. After coding and compiling the data, interesting trends were observed. The hope is that by better understanding the results and trends of trace DNA profiles in casework, crime laboratories can gain insight to better approach and prioritize different types of trace DNA evidence.

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### DNA, Trace, Case Study

## B57 Developmental Validation of the Quantifiler™ Trio Assay for the QuantStudio™ 7 Flex System

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**Learning Overview:** After attending this presentation, attendees will understand how to adapt standard Quantifiler™ Trio run and data analysis methods for use with an alternative real-time Polymerase Chain Reaction (PCR) system.

**Impact on the Forensic Science Community:** This developmental validation will enable laboratories to internally validate and implement the Quantifiler™ Trio assay on the QuantStudio™ 7 Flex system. Additionally, this presentation will impact the forensic science community by providing it with an alternative Quantifiler™ Trio run and data analysis workflow that can be used in place of the Human Identification (HID) Real-Time PCR Analysis Software.

The Quantifiler™ Trio DNA Quantification Kit is a commonly used assay for the detection, quantitation, and quality assessment of amplifiable human nucleic acid in forensic samples. The multiplexed, TaqMan™-based assay utilizes multi-copy loci for simultaneous detection of three human-specific genomic targets plus an internal PCR control. In combination, these components are used to: measure the total quantity of human DNA present in a sample, measure the quantity of male DNA present in a sample, evaluate human DNA degradation, indicate the presence of male-female mixture, and assess inhibition.

The Quantifiler™ Trio assay was designed and developmentally validated for the 7500 Real-Time PCR Instrument.<sup>1</sup> The newer QuantStudio™ 5 Real-Time PCR Instrument is also supported by the vendor for the assay, and can run the HID Real-Time PCR Analysis Software v1.3 that is marketed to simplify use of the Quantifiler™ quantitation assays for human identification applications. While this software is sold separately from the assay and instruments, the Quantifiler™ Trio assay user guide, support, and reference materials assume use of the software for run setup and data analysis.<sup>2</sup> The software cannot be installed on or run with alternate real-time PCR instruments.

This presentation will describe a developmental validation of the Quantifiler™ Trio assay with an alternate instrument, the QuantStudio™ 7 Flex Real-Time PCR System and a run and data analysis workflow that does not require use of the HID Real-Time PCR Analysis Software. The validation studies were performed in accordance with Scientific Working Group on DNA Analysis Methods (SWGDM) Validation Guidelines for DNA Analysis Methods and ISO 17025 method validation standards.<sup>3</sup> The workflow utilizes the instrument-standard QuantStudio™ Real-Time PCR Software v1.3 for run setup and raw data analysis, in combination with a custom Python® script to produce reports detailing run and sample quality metrics, plus the sample quantitation results. The Python® script is freely available and can be used or modified for use by any laboratory interested in performing Quantifiler™ Trio quantitation on the vendor-supported instruments or an alternative real-time PCR system.

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### Quantitation, Validation, Software

## B58 Establishing a Regional DNA Database

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**Learning Overview:** After attending this presentation, attendees will: (1) be familiar with the structure of a regional DNA database, (2) learn the challenges faced while implementing a regional DNA database, and (3) realize the importance of communication between local DNA databases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the implementation of a regional DNA database. The successes and challenges experienced during the deployment will be reviewed to assist other agencies in establishing additional regional databases, thereby enhancing communication among jurisdictions and maximizing the potential for case associations.

The Delaware Valley Investigative DNA Database (DVIDD) provides a regional DNA database for legally obtained DNA profiles, processed in either an accredited laboratory or through a validated Rapid DNA system. The DVIDD enhances collaboration in the region, establishes crime trends, and identifies persons of interest in criminal investigations.

The establishment of the DVIDD does not diminish the value of participation in the Combined DNA Index System (CODIS). All participants have been informed that CODIS is the primary and most robust source of DNA searching in the country, and whenever possible, samples should be processed for inclusion in CODIS. Currently, the Philadelphia Police Department (PPD) is a CODIS-participating laboratory within the Commonwealth of Pennsylvania and maintains a local DNA database though CODIS. The counties surrounding Philadelphia successfully maintain their own local DNA databases for crimes that are not entered into CODIS due to various procedural and resource constraints. Prior to the establishment of the DVIDD, these databases operated as standalone investigative tools despite the close proximity of the investigating agencies.

The DVIDD is structured as a non-CODIS DNA database to serve as a complementary tool for the regional partners to utilize the legally obtained and properly processed profiles that otherwise would not be searched against each other. As designed, profiles from the PPD local DNA database and local DNA databases of other participating members are routinely exported to the DVIDD. The PPD maintains operational custody and Quality Assurance/Quality Control (QA/QC) oversight of the DVIDD. Following a case association, the PPD reports the information to the appropriate agencies and advises on any confirmatory processes that may be needed.

The deployment of the DVIDD included stakeholder meetings and formal agreements between the county agencies to participate in the program. In these planning stages, the overall concept of the database was established, defining the roles and responsibilities of each agency. The implementation phase included developing profile acceptance criteria, search rules, and communications and reporting procedures. Following the deployment, a review of the successes and challenges experienced during the project was conducted to determine best practices that could aid in the establishment of regional databases in other jurisdictions.

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### Regional, DNA, Database

## B59 The Correlation of Hair Morphology and Length to Mitochondrial DNA (mtDNA) Massively Parallel Sequencing (MPS) Results

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**Learning Overview:** The goal of this presentation is to provide information on how the physical characteristics of a hair shaft, including the length of the tested hair, impact the success of obtaining mtDNA sequence profiles when using an MPS approach. Microscopical analysis of hairs prior to sequencing is typically not performed to assess whether morphological features contribute to the outcome. In addition, forensic laboratories often receive small hair fragments for analysis (<1cm), which limits the amount of testing that can be performed when using the conventional Sanger-Type Sequencing (STS) workflow. Attendees will learn about a newly developed hair extraction protocol which, along with a mitogenome MPS approach, can overcome many of the challenges.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a “morphology calculator” for determining the relative expectations of successfully generating mitogenome sequence data from a given hair.

Hairs are one of the most common evidence types found at crime scenes, and the extraction of both nuclear DNA (nucDNA) and mtDNA has been demonstrated.<sup>1,2</sup> Hairs in the anagen and catagen growth stages present follicular tissue, especially when forcibly removed, making them providers of nucDNA profiles when the roots are present. Telogen hairs are easily shed, and due to keratinization, leading to degradation of cellular organelles and nucleic acids, nucDNA is too degraded for analysis.<sup>2,3</sup> Therefore, mtDNA sequencing is the method of choice when analyzing shed hairs or fragments of hair shafts.

Historically, DNA testing has focused on recovering the control region of the mitogenome using electrophoretic techniques such as STS.<sup>1</sup> Haplotypes generated from the control region are generally good enough for discriminatory purposes; however, expanding to the entire mitogenome increases the discriminatory power.<sup>4</sup> Sequencing the mitogenome using STS is labor-intensive and a low-throughput method. Contrary to this, MPS analysis of the mitogenome can be performed relatively quickly with higher sample throughput. This study considered the effects of physical hair characteristics, including diameter, presence or absence of a medulla, and length tested, on mtDNA recovery and MPS data quality.

Donated hairs, ranging from 1-45 years of age, were sampled (0.5 and 0.1cm cuttings) starting at least 1cm from the root end, with some cuttings taken at various (mostly unknown) lengths for hairs without a root. The fragments were characterized microscopically prior to DNA extraction, which was performed using a modification to a previously published protocol, and copies of mtDNA were quantified using a custom quantitative PCR (qPCR) assay.<sup>3</sup> The PowerSeq™ Whole Mito System prototype kit was used for amplification, followed by preparation of libraries using the Illumina® TruSeq® DNA PCR-Free HT Library Preparation kit. The Illumina® MiSeq® instrument and 600 Cycle MiSeq® Reagent Kit v3 was used for sequencing, and the sequencing data was analyzed using GeneMarker® HTS from SoftGenetics®. A “morphology calculator” was developed for practitioners and researchers to use that will assist in determining the relative success rates for generating complete mitogenome haplotypes from small hair fragments with different physical characteristics.

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### Next Generation Sequencing, Forensic, Hairs

## **B60 An Assessment of the NovaQUANT™ Human Nuclear and Mitochondrial DNA Quantitative Polymerase Chain Reaction (qPCR) Assay Using the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2372a**

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**Learning Overview:** The goal of this presentation is to inform attendees of an evaluation of the NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Kit utilizing the NIST SRM 2372a.<sup>1,2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing qualities of an accurate and robust real-time qPCR assay and how the SRM 2372a can be used to verify the concentration of DNA standards used in absolute qPCR assays. In turn, downstream analysis failures can be minimized, particularly when valuable casework samples are limited.

In forensic DNA casework, a highly accurate real-time qPCR assay is recommended per the Scientific Working Group on DNA Analysis Methods (SWGDM) to determine whether the DNA is of sufficient quantity and robust quality to move forward with downstream Short Tandem Repeat (STR) or sequencing analyses. One fundamental issue with absolute qPCR is that the quantifiable concentration of the commercial assay standards can vary depending on origin (i.e., whether from a cell line or a human subject, supplier, lot number, shipping method, etc.). In 2018, NIST released a human DNA standard reference material for evaluating qPCR quantification standards, SRM 2372a, which contains three well-characterized human genomic DNA samples, including (A) a single male<sub>1</sub> donor, (B) a single female<sub>1</sub> donor, and (C) a 1/3 male<sub>2</sub>/female<sub>2</sub> donor, each with certification data for nDNA and as well as for mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratios.<sup>2</sup>

The SRM 2372a was used to evaluate the commercial qPCR assay NovaQUANT™, which amplifies four targets, each in separate singleplex SYBR® Green qPCR assays: two mtDNA gene targets ((ND6 (96bp) and (ND1(153bp)) and two nDNA targets ((BECN1(129bp) and (NEB (116 bp))).<sup>1</sup> NovaQUANT™ was performed using the absolute standard curve method utilizing osteosarcoma cell line 143B DNA.<sup>1</sup> The data was evaluated using the Minimum Information for Publication of Quantitative Real-Time Experiments (MIQE) guidelines.<sup>3</sup> While the data for all four targets for qPCR efficiency (perfect doubling of product with each cycle) and correlation coefficients ( $r^2$ ) values fell within the MIQE guidelines, the quantification accuracy, which was affected by sensitivity and specificity, was less than optimal. The results showed that all three SRM 2372a components (i.e., A, B, and C) quantified higher than their expected NIST values.<sup>2</sup> A second analysis, not reliant on the 143B DNA standard, was conducted using the NovaQUANT™ Relative Copy Number Method.<sup>1</sup> Those results showed that SRM 2372a quantified much more accurately compared to the absolute quantification method. Therefore, the inaccuracies associated with absolute quantification may be due to quantitative imprecision and/or qualitative issues of the cell line 143B DNA standard itself.

In conclusion, the availability of a commercial qPCR assay like NovaQUANT™ that can quantify mtDNA and nDNA simultaneously is a valuable tool for forensic DNA analysts, potentially saving time and reducing cost. However, when utilizing an absolute qPCR method with a standard, such as 143B cell line DNA, the standard should be verified using a well-characterized DNA standard reference material like the NIST SRM 2372a to ensure high qPCR accuracy and success.

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### **NovaQUANT™ qPCR, NIST SRM 2372a, Absolute Quantification**

## B61 The Visualization of Seminal Stains on Dark Textiles

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**Learning Overview:** After attending this presentation, attendees will understand the difficulty with traditional screening processes for human seminal stains on dark textiles and potential approaches for a higher success rate of the visualization of stain patterns

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying information about the search and visualization of latent seminal stains on dark textiles. Potential techniques for improving the successful visualization of seminal stains on dark materials will be discussed in this presentation.

Searching latent seminal stains during a crime scene investigation can often be achieved using an Ultraviolet (UV) light or an Alternate Light Source (ALS) with a combination of filters. Followed by a chemical test, a crime scene investigator will collect and preserve the evidence for further analysis. However, false-negative outcomes have been found due to the lack of fluorescent emission from the seminal stains on a dark textile. The crime scene investigators must recognize the potential factors associated with false-negative results.

In this project, known human seminal fluids were deposited on different types of dark textiles. Thirty-eight different types of dark textiles were used, consisting of 100% polyester, various polyester blends with spandex, nylon, cotton, acetate, and wool, 100% cotton, various cotton blends with spandex and polyester, nylon with rayon and lycra mixes, and 100% suede. Twenty-seven were laundered before adding the sample, and 11 were unlaundered and sampled as purchased. Controlled samples were prepared by adding 1–2 drops of semen from a pipette to the center of the fabric. All stains were dried for a week. Several commonly used standard visualization methods were used for the visualization of known seminal stains on dark textiles. The visualization methods were: (1) a UV light, (2 and 3) two types of flashlights (455nm) for excitation with an orange filter for fluorescence observation, and (4) a laser (532nm) for excitation with an orange barrier filter for fluorescence observation. Of the total of 38 control samples, it was found 71% of stains were visible to the naked eye, with 53% (20 samples) showing clear deposit patterns. Using traditional UV imaging with no filter, the percentage of visible stains was 58%. Using an ALS at 455nm coupled with an orange filter, successful visualization of seminal stains ranged from 21%–61%, depending on the excitation light sources. The laser at 532nm with an orange filter had a success visualization rate of 34%. Four fabric samples showed false negatives with all visualization techniques, two of 100% polyester blend, one laundered and one not laundered, one not laundered 95% polyester/5% spandex blend, and one laundered 65% polyester/35% cotton blend.

The next step in the lab is to adopt a hyperspectral imager to improve the success rate for the visualization of seminal stains on dark textiles. The goal is to use multiple wavelengths to negate the background quenching effect that the textile has on the stain. The primary focus will be on polyester and polyester blends, as they were the only samples that all methods resulted in a false negative.

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### Crime Scene Investigation, Alternate Light Source, Seminal Stains

## **B62 Indirect DNA Transfer—A Murder Case With Unexplained DNA Traces on a Spent Cartridge Case**

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**Learning Overview:** After attending this presentation and reading about this murder case and the research ideas regarding indirect DNA transfer on spent cartridge cases, attendees will have gained more information about this frequently and intensely discussed relevant topic in forensic genetic research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that indirect DNA transfer is currently a highly analyzed and cited topic in forensic genetic research. Further research in this field is needed, and the publication of cases where indirect DNA transfer occurred and complicated criminal investigations is of the utmost importance.

In 2019, the murder of a 20-year-old woman in Zell am See, Salzburg, Austria, was committed. Her neighbors found her shot and barely alive in front of her apartment door in the stairwell of a mid-sized apartment building, where she succumbed to her injuries not much later. The murderer was eventually found on detours and through coincidences, and he was finally convicted one year later after making a partial confession to the fatal shooting. However, his DNA-profile could not be found on the crime scene even though in total 25 useful traces could be secured and were processed, some of them even leading to DNA database hits.

Thus, there were numerous useful DNA traces at the crime scene and after analyzing those, there were no DNA profiles unaccounted for. Especially interesting was one specific trace: a precise female profile of a woman who was eventually ruled out as a suspect was found on one spent cartridge case lying inside her apartment, close to the front door. This woman was acquainted with the victim and was occasionally at her apartment but was not involved in the actual shooting and was not present when it happened. So how did this DNA profile end up on the cartridge case with a very strong and good profile quality without her being involved in the murder? In this case, based on DNA analysis alone without the coincidental finding of other indicators leading to the conviction of the real murder, this woman would have stayed the lone suspect.

In this study, based on this murder case involving likely indirect DNA transfer, the aim is to find more answers regarding this topic. Therefore, this study plans the evaluation of diverse scenarios as to how DNA could be transferred on cartridge cases without the donor present, before and after shooting a weapon.

A research project is needed to possibly find additional answers for this specific murder case from Salzburg and to improve the understanding of the mystery of indirect DNA transfer in the context of spent cartridge cases in general.

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### **DNA Transfer, Cartridge Cases, Forensic Genetics**

## B63 A Multivariate Statistical Approach for Enhancing Ancestry Prediction From Microhaplotype Data

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**Learning Overview:** After attending this presentation, attendees will be able to appreciate the advantages of using Multivariate Data Analysis (MDA) techniques on Microhaplotype (MH) data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternative statistical approach to infer Biogeographical Ancestry (BGA) at higher resolution compared to conventional Principal Component Analysis (PCA) methods that are commonly used in forensic routine analysis.

MHs are newly developed multi-allelic markers of at least two Single Nucleotide Polymorphisms (SNPs) within < 300bp. Due to the presence of small amplicons and a low recombination rate, absence of stutter and preferential amplification, they are promising candidates for human identification, mixture deconvolution, and BGA prediction. The global interest in BGA inference aims to maximize the amount of forensically relevant information retrievable from DNA evidence. Current protocols for ethnic origin estimation are generally based on PCA and Bayesian approaches. Early studies demonstrated the capability of multivariate statistical tools to predict the ancestry affiliation from autosomal Short Tandem Repeat (STR) data. This study aimed to extend this alternative and dynamic statistical approach to the analysis of MH data.

Multivariate techniques such as Partial Least Squares Discriminant Analysis (PLSDA) and Support Vector Machines (SVM) were used for the analysis of MH profiles from an initial set of 347 individuals from four United States population groups (88 African American [AA], 114 European-American [EA], 102 Southwest Hispanic [His], and 43 East-Asian American [EAA]). The DNA samples were typed using a bioassay of 74 MHs on the Ion S5™ sequencing platform. Overall both PLSDA and SVM approaches significantly improved ancestry inference by enhancing the separation of the four population clusters. Furthermore, PLSDA results were used to assess the accuracy of ethnic affiliation for four test individuals, one per each reference population group, to test their ancestry affiliation in terms of Likelihood Ratio (LR). The resulting LR values provide an indication of how much more likely it is to observe the MH profile of interest if it originated from the test population at the numerator than if it originated from the other three populations at the denominator. LR values for each of the four test individuals confirmed the correct ancestry affiliation and, in particular,  $LR = 10^{99}$ ,  $LR = 10^{62}$ ,  $LR = 10^4$ , and  $LR = 10^1$  were obtained for AA, EAA, EA, and His, respectively. Overall, high LR values were observed for well-genetically defined populations while lower values from admixed ones, as expected.

The multivariate statistical approach will be shortly implemented in an open-source and user-friendly R Shiny app to assist law enforcement agencies and forensic DNA analysts with the interpretation of BGA data.

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### Microhaplotypes, Biogeographical Ancestry, Multivariate Data Analyses



## **B64 The Biogeographic Origin and Genetic Characteristics of the Peopling of Jeju Island Based on Lineage Markers**

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**Learning Overview:** After attending this presentation, attendees will understand the biogeographic origin and genetic characteristics of a relatively isolated population within Korea residing in an island apart from the mainland of Korea called Jeju Island and the power of lineage markers to infer the genetic origin or characteristics of populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting genetic evidence for an isolated population within Korea reflecting its historical background and geographical location, which is important to be considered in a forensic application.

Jeju Island is the largest island of Korea, located southwest of the Korean Peninsula, and has built a unique history and culture distinguished from other provinces within the mainland of Korea. Jeju Island has actively interacted with neighboring countries, probably due to its geographical location of the central region of East Asia, and also has been influenced by the Northeast regions, such as Mongolia. In various studies, much evidence of Mongolia's influence on Jeju Island has been found in their language, customs, and food, but little research has been conducted on genetic approaches so far. Lineage markers on Y-chromosome and mitochondrial DNA are useful in inferring paternal and maternal lineages of populations, respectively, and can be of help in understanding the biogeographic origin of peopling of Jeju Island.

This study analyzed data of Y-chromosomal Short Tandem Repeats (Y-STRs) of 615 males and mitochondrial DNA haplogroups of 799 individuals in Jeju Island using statistical methods, including pairwise Rst and Fst, Analysis of Molecular Variance (AMOVA), and Metric Multidimensional Scaling (MDS) analyses, with collected reference data of Korean populations within different provinces and other countries.

The results from Y-STR analysis presented that low diversity of Y-STR haplotypes, strong association with surnames, significant genetic difference among other males within different provinces of Korea, and common genetic variations known to be predominant in Mongolian people. Statistical analysis of mitochondrial DNA haplogroup showed that low diversity of haplogroup and high frequency of haplogroup Y was mostly prevalent in ethnic populations around the Sea of Ohkotsk in Northeastern Asia. All these results could indicate that a genetic substructure of Jeju Island may exist, possibly due to genetic drift acting on this somewhat isolated island. In addition, it is believed that the peopling of Jeju Island may be genetically influenced by northern regions such as Mongolia, which can be supported by the fact that Jeju Island has historically had a close relationship with the Yuan Dynasty established by a leader of the Mongol people during the Koryo Dynasty of Korea. The present study provided genetic evidence on a unique background of peopling in Jeju Island distinguished from the mainland of Korea, and this should be taken into account for forensic application, including the establishment of forensic databases for disaster victim identification, such as the Jeju 4.3 incident.

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### **Jeju Island, Y-STR, Mitochondrial DNA Haplogroup**

## **B65 The Optimization of DNA Extraction From Buccal Samples Collected With the CollectEject™ Oral Swab for High-Throughput Testing**

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**Learning Overview:** After attending this presentation, attendees will be informed of a new collection product that can be utilized for collection of reference samples, Genturi's CollectEject™ Oral Swab. Attendees will also learn two optimized extraction procedures to produce maximum yield from the CollectEject™ Swabs and how it compares to other common collection devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into various collection devices that can be used to streamline processing of reference DNA samples as well as two optimized extraction techniques that maximize DNA yield with the CollectEject™ Oral Swabs for a high-throughput workflow.

As the submission of casework samples to be processed for DNA increases, and a newer panel of testing options becomes available for law enforcement (e.g., Rapid DNA or genetic genealogy), crime scene processors and laboratories look for ways to streamline collection and testing procedures to keep up with this demand. Collection techniques and devices continue to evolve to allow samples to be collected more efficiently without compromising integrity or contamination of the sample. Genturi has recently released a product line of swabs, CollectEject™, that allows for a quick and efficient collection process. The swab is attached to an applicator that ejects the tip of the swab straight into a tube with no cutting necessary. The swab design also allows for increased surface area for DNA collection and retention. Newer technologies and workflows rely on high first-pass success, like Rapid DNA devices, or newer investigative lead processes like genetic genealogy or phenotyping that require higher quality and quantity DNA for investigative leads. The collection techniques utilized for these processes should be quick and of high quality.

This research evaluated the CollectEject™ swab for collection of reference samples. The first study focused on optimizing the yield of DNA extracted from the CollectEject™ swab following two different DNA extraction protocols, PrepFiler® Express using the AutoMate Express™ System and the Promega® Maxwell extraction. Various incubation times and temperatures were analyzed and compared by quantitation of total DNA extracted. A statistical analysis was performed on the yield of DNA recovered through each variable, and the optimal parameters for each extraction process was determined. The second phase of the study compares the CollectEject™ swab to the Whatman® Omni Swab. Reference swabs were collected using each device and stored in Genturi's SwabSaver™. The SwabSaver™ allows for samples to be stored at room temperature for a prolonged periods of time and the initial extraction stage can be performed directly in the SwabSaver™ tube. Samples were then extracted following the optimized Automate extraction procedure and quantified for comparison. A select number of samples will be carried through to amplification to ensure there is no effect on the quality of DNA profile generated.

Quantitation results of the first study were separated and analyzed by extraction type. For the Automate Express™ extraction, the 80-minute incubation mixed at 850RPM at 70 °C was found to yield significantly higher total DNA compared to other parameters tested. The 80-minute extraction yielded an average of 7,261pg of DNA and the remaining variables ranged from 963 to 2,230pg total DNA. All other variables tested generated no significant differences. The Maxwell extraction yielded no significant difference between incubation temperatures (56–70°C) and incubation times (30 minutes–12 hours). A higher sample size and DNA profile generation will be completed to further examine compare these devices.

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### **DNA Collection, CollectEject™, STR Profiling**

## B66 One Swab, Two Uses: Rapid DNA and Conventional DNA Testing

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**Learning Overview:** The goal of this presentation is to show that testing a swab with a rapid DNA device does not consume all of the DNA on the swab.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by removing the fear of sample consumption that is always present when running lower level DNA samples.

One of the concerns with rapid DNA testing has always been sample consumption. Sample consumption often requires permission from attorneys in order to prevent court challenges by the defense. As a result, it is important that whatever sample is used to generate an investigative lead from a DNA sample does not consume all of the sample. Rapid DNA, which can be used to develop a DNA profile at a scene in under two hours, can be used to generate important investigative leads. However, if the DNA is consumed by the rapid DNA instrument, it prohibits additional DNA testing using conventional forensic laboratory methods. To address this problem, studies were performed to determine if the same swab run on the Applied Biosystems™ RapidHIT™ ID System can be removed and used again with conventional DNA techniques to achieve similar quality profiles.

The extraction methods typically used in forensic DNA laboratories are designed to remove as many cells from the substrate as possible. The methods frequently involve long incubation times and vigorous shaking of the samples. With rapid DNA instruments, the extraction methods are more passive in nature and do not involve shaking of the sample. Based on this, it is feasible that after a sample goes through extraction on the RapidHIT™ ID System, there may be sufficient cellular material remaining on the swab to enable successful DNA profiling using conventional methods.

The goal of this presentation is to show that testing with the RapidHIT ID™ does not consume all of the DNA on the swab. For certain sample types, a DNA profile can be generated twice from the same swab—once for an investigative lead and then again for court purposes if needed. The ability to obtain DNA profiles twice from the same swab will give law enforcement the confidence to use this technology to generate much needed investigative leads, knowing that they still have sample preserved for future testing if it became necessary.

The RapidHIT™ ID System, unlike some other rapid DNA instruments, allows for the easy removal of swabs after they are processed. Using conventional DNA techniques of extraction, quantification, amplification, and capillary electrophoresis on high- and low-level DNA samples, it was determined that there is in fact enough cellular material left over after the swab was run on the RapidHIT™ ID to produce a full DNA profile. The sample types tested included buccal swabs at varying amounts of swipes in the mouth, blood samples at varying volumetric levels, and crime scene type samples such as cigarette butts and drinking containers. In all cases, a DNA profile was achieved on the RapidHIT™ ID and after the swab was removed and processed using conventional DNA techniques.

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### Investigation, Rapid DNA, Consumption

## B67 The Identification of Various Body Fluids Obtained From the Crime Scene With Fourier Transform Infrared (FTIR) and Age Determination

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**Learning Overview:** After attending this presentation, attendees will have learned about the detection of body fluids (saliva, blood, semen, vaginal fluid, and urine) by FTIR and its advantages

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling the determination of body fluids with FTIR and by giving a chance to advanced analysis since it doesn't have any sample loss.

Forensic science is a wide research field, which aimed at developing analytical methods to analyze evidence to ensure justice through criminal investigation. Identification of body fluids such as blood, semen, saliva, vaginal fluid, etc. should be done correctly since determination of time-dependent changes in these fluids and obtaining information about the time they were at the scene are very important in illuminating forensic cases. In addition, the development of DNA analysis requires proper collection and preservation of body fluids.<sup>1</sup> Many stains of body fluid are invisible, present in very small quantities or in a mixture, and therefore not always easy to identify. Identification of body fluids allows one to determine the biological source of stains, as well as conducting DNA analysis and interpreting their results. Identification of body fluids in forensic cases is generally evaluated by enzymatic and immunological tests as a screening test at the scene.<sup>2</sup> One of the important disadvantages of these tests is that they have margins of error and create cross-reactions between other species and tissues, since most of them are not specific to identify species. In addition, there is no improved enzymatic or immunological tissue test for vaginal secretion identification. These disadvantages have led to the development of IR spectroscopy, which is a non-destructive, fast, and confirmatory analyzation method.<sup>3</sup>

FTIR is emerging as the preferred method of analysis for non-destructive analysis of the chemical composition of unknown biological stains. FTIR has the ability to do preliminary identification and further studies to determine the age of unknown biological stains at crime scene and even make interspecies distinction. In addition, this method is advantageous over most existing biochemical tests due to its high specificity, universality, non-consumable nature, and allowing samples to be examined directly over the substrate in crime scenes.<sup>4</sup>

In this presentation, a new overview of applications of FTIR for forensic analysis of body fluid traces is presented. Body fluid samples were analyzed by taking on various substrates and exposing them to certain environmental conditions in order to simulate scenarios related to forensic events. Nine different parameters (fresh, 1 hour, 3 hours, 1 day, 3 days, 1 week, 15 days, 1 month, and 1 year waiting samples) were determined according to the time and were examined over five different body fluids, namely saliva, blood, semen, vaginal fluid, and urine. Samples taken from ten volunteer women and ten male individuals were stained on fabrics, which were not used before, and exposed to ultraviolet radiation for two hours after being washed in the washing machine at 90°C. After that, these staining samples were analyzed after being dried at 25°C (room temperature).

As a result of this study, samples were identified using FTIR spectroscopy without consumables instead of serological tests. In addition, age determination was made by using time-related changes in the chemical components in the body fluids.

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2. Makhni T., Ilchenko O., Reynat A. et al. Age-Related Changes In FTIR And Raman Spectra of Human Bloodpacs. *Atoms and Molecules*. 2016;61(10).
3. Takamura A., Watanabe K., Akutsu T. Soft and Robust Identification of Body Fluid Using Fourier Transform Infrared Spectroscopy and Chemometric Strategies for Forensic Analysis. *Scientific Reports*, 2018; 8:8459.
4. Wei X., Yu K., Wu D. Species Identification of Semen Stains by ATR-FTIR Spectroscopy. *International Journal of Legal Medicine*, 2020.

### Forensic Sciences, FTIR, Age Determination

## **B68 The Stability of Semenogelin-I (Sg-I) in Seminal Fluid and Seminal Stain on Various Fabrics Under Various Temperatures**

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**Learning Overview:** After attending this presentation, attendees will better understand information about the stability of semenogelin, especially Sg-I, in human seminal fluid as well as in seminal stains on three types of fabrics (cotton, polyester, and Thai silk) under five different temperatures (-80°C, -20°C, 4°C, 25°C, and 37°C) and nine different time points up to 180 days. This essential basic knowledge can fill the current gap in intelligence about using the Sg-I as a semen biomarker for semen identification to solve any specific forensic issues of sexual assault cases in their daily practice or future research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as essential knowledge of Sg-I stability in seminal fluid and seminal stains on various fabrics under various temperatures and over different time periods for semen identification in forensic application, especially in rape cases, that has been challenging the forensic community worldwide.

Sexual assault is one of the most acknowledged offenses occurring worldwide, including in Thailand. A successful prosecution of this criminal act needs proof of semen detection. Although sperm identification is essential for seminal investigation as well as assailant identification, its absence may be found in certain cases. Appropriate biomarkers of semen, especially Semenogelin (Sg), are then tested for confirmatory semen identification. Sg contains two forms; Sg-I (52kDa) and Sg-II (71 and 76kDa). Sg-I is the major component of the seminal plasma and the main component of the human semen coagulum. However, Sg-I concentration in seminal fluid varies according to environmental temperature and duration. Little has been known about its stability in the fluid under various conditions. This study was thus aimed at investigating the stability of Sg-I in ejaculated seminal fluid as well as in seminal stain on three types of fabrics (cotton, polyester, and silk) under five different temperatures (-80 °C, -20°C, 4°C, 25°C, and 37°C) and nine different time points up to 180 days using enzyme-linked immunosorbent assay.

The results indicated that Sg-I concentrations either in seminal fluid or in seminal stain declined significantly over time periods and under all temperatures studied. At -80 °C and -20 °C, Sg-I in seminal fluid was still detectable on day 180, approximately 70% and 40% of its original concentration, respectively. At 4°C, 25°C, and 37°C, Sg-I in seminal fluid was still detected on day two but not on day three. The Sg-I in seminal stains was still detectable on day 180 under all temperatures investigated with subsequent lower concentrations. Among the studied fabrics, silk was the most Sg-I-preserved fabric.

In summary, the concentrations of Sg-I either in seminal fluid or in seminal stains were higher at lower storage temperature and in shorter time periods. The Sg-I in seminal stain was much more stable than that in seminal fluid under all temperatures studied. The information obtained from this study would provide a significant insight to forensic scientists for better understanding and more appropriate handling of their case works.

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**Rape, Semen Marker, Seminal Vesicle-Specific Antigen**

## B69 Human Identification From Washed Semen Stains

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**Learning Overview:** After attending this presentation, attendees will understand that violence in a society may exhibited be in various ways. The most important evidence of physical violence is biological evidence, such as blood, semen, saliva, hair, and nails. Blood and semen stains are the most common evidence of violence against women. The most important evidence to find the suspect of sexual assault are semen stains. In many studies, it has been observed that rapists do not use condoms in many cases and transfer seminal fluids over the body of the victim, to their own clothes, bed linen, or other objects in the environment.<sup>1</sup> This reveals the necessity of examination of the bed linen and the victim's underwear during crime scene investigations. However, in many cases, it has been seen that the rapists and the victims tend to clean their bodies and clothes because of psychological conditions such as fear, panic, and anxiety. They may even hide the underwear for a long period of time.<sup>1</sup> This leads to the loss of hope for the victims who could not call upon the judicial units immediately after the incident due to several reasons and decided to complain later.

**Impact on the Forensic Science Community:** The victim who suspects the results obtained from the authorities may tend to hide the clothes and may not hand the physical evidence to the laboratory.<sup>2,3</sup> However, recent studies show that DNA can be obtained from clothes that are washed more than once in different temperatures.<sup>2</sup> Due to the characteristics of seminal fluid and the fact that sperm cells get stuck in fabric fibers, DNA can be obtained after clothes have been washed more than once. The amount of DNA isolated may differ depending on the type of cleaning reagent that was used, type of stained fabric, temperature, and drying conditions as well as the preservation period.<sup>1-3</sup> This presentation will impact the forensic science community by demonstrating whether DNA can be obtained from washed underwear to evaluate the amount of isolated DNA and by showing if identification can be made with the obtained DNA.

In this study, both cotton- and nylon-blended fabrics were stained with semen samples taken from volunteers. Semen stains on the fabrics dried at room temperature were washed with detergent at 40°C, 60°C, and 90°C and dried again at room temperature. The washed and dried pieces of fabric were examined by Ultraviolet (UV) light source and stain residues were made re-visible. After this step, DNA was isolated from the stained part of each washed fabric by two different isolation methods.

As a result of this study, comparisons could be made on DNA isolation methods, washing conditions, and how much DNA could be obtained on different washed fabrics. The limits in which conditions for washed semen stains are sufficient for DNA analysis are drawn.

In conclusion, this study reveals the necessity of more detailed and careful studies on the washed laundry sent to criminal laboratories for examination. It gives hope to the victims who have not been able to go to judicial units immediately after the incident because of fear or for any other reason, and who think that they cannot get results because they have washed the laundry. This also sheds an important light in the interest of justice.

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### Forensic Serology and Biology, Human Identification, Washed Semen Stains

## B70 “The Sexome”: Identifying Unique Microbial Signatures in Male and Female Pairings

*Natasha Nye, MS\*, Western Australia Police, Yangebup, Western Australia 6164, AUSTRALIA; Brendan Chapman, BSc\*, Forensic Science, Murdoch University, Perth, WA 6150, AUSTRALIA*

**Learning Overview:** The goal of this presentation is to inform attendees of sexual assault case relevance and confidence in the determination of a sexual act.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by developing scientific confidence in sexual assault case reports in determining that a sexual act has occurred, even without the presence of spermatozoa. This could also prove fundamental to sexual assault research to cases in children who are subject to frequent assault by family members.

Sexual assault casework typically involves analysis following the collection of a vaginal sample. The presence of spermatozoa in a vaginal sample exhibits as viable proof that a sexual act has occurred. Resulting analyses may also indicate the identity of the assailant by means of profiling the biological DNA present in a sample. However, the presence of spermatozoa can deteriorate over time or be completely absent from a mixed sample due to a number of factors such as the assailant is aware that the deposit of seminal fluids can implicate in a possible profile. Another factor to consider is that there may be, by chance, a complete absence of spermatozoa in the seminal sample due to a possible vasectomy or a condition of azoospermia or oligospermia. Ultimately, each circumstance would likely result in a profile that is unable to be determined. Current research methods are examining the human microbiome as a means of profiling by the identification of unique bacterial signatures in specific areas of the body. Recent studies are attempting to determine the typical bacteria in the urogenital microbiome for means of identifying male- and female-specific species of bacteria. The aim of this particular study is to examine the urogenital microbiome of male-female pairings to identify unique bacterial signatures, and if these signatures transmit following intercourse.

There were 10 consensual heterosexual couples who participated in the study (20 participants in total). Each couple were instructionally directed to sample their own genitalia prior to and following intercourse. Pre-coital samples were sampled three to four days post-menses (for females). Post-coital samples were collected approximately two to six hours following intercourse to best replicate typical reporting periods for sexual assault victims. These samples were extracted using a PureLink™ Microbiome DNA Purification Kit and subsequently sequenced by the 16S Ribosomal RNA Gene Amplicons for the Illumina® MiSeq® System to identify sex-independent, unique bacterial signatures in males and females and subsequent transmission. Sequenced data was analyzed by the MiSeq® Reporter software for classifications of organisms from the V3 and V4 amplicon via a 16S rRNA database. This classification is based on the Greengenes database and the output was read at each taxonomic level to identify unique bacteria in the male and female microflora.

While human microbiome research is in its relative infancy, future studies should be conducted to understand and discriminate the male and female microbiomes. In particular, examining the unique microflora of their urogenital microbiome. It would develop the scientific confidence in sexual assault cases to determine that the sexual act has occurred. Additionally, future studies could improve the capability of detection of male bacteria in female sexual assault victims, ultimately resulting in enhanced profiling methods and confidence for females in reporting sexual assaults. This particular research would also serve well for sexual assault cases where male DNA fractions are not detected via typical analyses. It could also prove fundamental to sexual assault research to cases in children who are subject to frequent assault by family members.

### Sexual Assault, Microbiome, Bacteria

## B71 The Development of Epigenetic Methylation Markers for Skin/Sweat

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**Learning Overview:** After attending this presentation, attendees will have gained valuable insight on the research efforts being made to use DNA methylation patterns at different genome locations as a tool to identify skin cells.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by determining skin/sweat from other body fluids.

The determination of tissue type is important when reconstructing a crime scene. However, at present there is no specific methylation-based marker to distinguish touch DNA from other body fluids. Because epigenetic markers capable of identifying sweat and/or skin have not yet been developed, the epigenome is being explored to discover loci of interest. Typically, the DNA in such samples results from free DNA secreted in sweat and cellular material and may result from skin sweat or other catabolic processes. Work in the lab has successfully identified DNA methylation in various body fluids, but not in skin or sweat. Moreover, the development of a body fluid identification multiplex in the lab would greatly benefit from the inclusion of a skin/sweat marker. The ultimate goal is to develop multiplexed methylation-based epigenetic loci for use in forensic analysis.

This study compared DNA obtained from skin with semen, saliva, blood, and vaginal epithelia. An Illumina® MethylationEPIC Beadchip Array was utilized to perform an Epigenome Wide Association Study (EWAS) to determine suitable methylation markers for touch DNA. The results indicated that CpGs from five different genes were potentially of use: WDR11, PON2, NAA16, MRPS15, and NHSL1. A set of Polymerase Chain Reaction (PCR) primers were designed to encompass these loci. Next, DNA was extracted, quantified, and bisulfite converted using the QIAGEN® EpiTect™ Bisulfite Kit to convert all the unmethylated cytosine to uracil on the DNA template. Bisulfite-converted DNA was amplified using the Rotor-Gene® SYBR® Green PCR Kit on a Rotor-Gene® Q, then analyzed for melting temperature.

This high-resolution melt analysis was then used to examine various epigenetic loci with potential for the discrimination of touch DNA. In these studies, several loci showed bisulfite-modified skin amplicons that melted at lower temperatures when compared to blood, saliva, semen, and vaginal epithelia. These results demonstrate that High Resolution Melt (HRM) analysis may be a promising technology to identify skin/sweat from other body fluids of a DNA sample.

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### DNA Methylation, Skin/Sweat, High Resolution Melt



## B72 The Evaluation of Degraded Human DNA Samples Using the Illumina® Global Screening Array

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**Learning Overview:** After attending this presentation, attendees will better understand the analysis limitations of genome-wide Single Nucleotide Polymorphism (SNP) genotyping data from low-quantity and degraded forensic samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results for considering alternative data metrics for assessing the accuracy potential in downstream analyses and forensic genealogical investigations as it applies to degraded DNA.

Forensic Genetic Genealogy (FGG) applies enhanced genetic processing techniques (array-based genome-wide SNP genotyping) coupled with traditional genealogical research techniques to generate new investigative leads. Application of FGG technology can be most useful 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.

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. Previous work to optimize sensitivity of the assay demonstrated a total DNA input <1.0ng successfully generated high-quality genotyping data.

An additional challenge this method faces with forensic samples, other than DNA quantity, is the quality. For SNP genotyping analysis, the quality of the data is critical. The goal of the degradation study is to evaluate how the severity of DNA degradation affects the quality of the SNP data.

The severity of DNA degradation in traditional Short Tandem Repeat (STR) analysis can be visualized as it presents an identifiable sloped pattern demonstrating the interruption of the polymerase during amplification. As the amplification chemistries are fundamentally different between STR and SNP typing, it is hypothesized that the form of DNA degradation could have less of an impact on the genotyping data.

For this study, genomic DNA was experimentally degraded using Ultraviolet C (UV-C) light at defined intervals up to 1.0J/cm<sup>2</sup>. Samples were quantified pre- and post-treatments and typed using GlobalFiler® to confirm degradation. The STR profiles coupled with the Degradation Index (DI) from the quant method demonstrated that as the dosage increased, so too did the amount of degradation (quantified via visual pattern in the profile and increase in DI). Five samples expressing specific degradation patterns based on STR results were selected for genome-wide SNP genotyping, in duplicate, on the iScan to evaluate the performance of the Infinium™ Global Screening Array in its ability to accurately type Low-Copy Number (LCN) degraded DNA sample types.

Analysis of the SNP data showed similar trends in most aspects. Concordance of the degraded samples did trend down as the dosage amount increased. Concordance was performed between treated samples and a control sample run alongside. One metric used to evaluate the performance of the assay is the call rate, which is a percentage of the total number of SNPs genotyped over the total number of SNP targets in the assay. When evaluating the samples using this metric, the trend was unexpectedly parabolic. As the dosage level increased, the curve trended down, then re-bounded and began increasing, resulting in the 0.0mJ/cm<sup>2</sup> and the 1.0J/cm<sup>2</sup> samples having very similar call rates.

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### SNP Genotyping, Forensic Genetic Genealogy, DNA Degradation

## B73 Evaluating the Use of the M-Vac® Wet Vacuum System to Recover DNA From Cotton Fabric

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**Learning Overview:** With the current state of the field of forensic biology, collecting touch DNA from evidence samples poses some difficulty. Some traditional methods for collection of DNA from biological fluids and touch samples include cuttings, scrapings, and the double swab method. While all of these methods have benefits, they also have their drawbacks. In order to more efficiently collect DNA from an evidence sample, novel approaches need to be explored. The goal of this presentation is to introduce attendees to this novel approach: the Microbial Vacuum (M-Vac®) Wet Vacuum System.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing this system that utilizes a sterile collection solution and a vacuum to collect DNA material from evidence samples. While it is similar to the double swab method, it takes collection a step further by collecting DNA material within the pores of samples. It is especially beneficial for cold cases where DNA material from the surface of a piece of evidence was previously collected, but there could still be trace DNA trapped in the pores. This method eliminates issues related to sample size and attempting to determine where to collect samples on a piece of evidence. It also has the benefit of sampling evidence areas directly and in their entirety. This can decrease the number of evidence samples needed to be transported to the laboratory for processing since the M-Vac® can be used at crime scenes. Therefore, this system is beneficial for its use as a serological tool for a crime scene analyst and a DNA analyst.

Some preliminary research has been performed by the Moroosse Research Lab comparing the M-Vac® to traditional cuttings for collection of blood, saliva, and semen and swabbing for touch DNA on unworn and 15-minute worn cotton t-shirts. The unworn study showed that the M-Vac® may be beneficial with the collection of touch DNA as compared to swabbing. As for the 15-minute worn study, it was shown that background wearer DNA has the potential to interfere with the interpretation of an evidence sample's electropherogram when collected with the M-Vac® by being preferentially amplified over DNA from an evidence sample. However, with t-shirts only being worn for 15 minutes, not much can be said about wearer interference.

So, to determine the extent interference-wearer DNA can have on an evidence sample's electropherogram when collected with the M-Vac®, a study with t-shirts worn for 12 hours was conducted. This study was the same as the previous worn study conducted with the only difference being that the t-shirts were worn for 12 hours instead of only 15 minutes. T-shirts were worn by individuals for 12 hours consecutively, then a biological fluid or touch DNA was placed onto the worn t-shirt. Fluids were either blood, saliva, or semen. This new study helped to fill the gap of knowledge from the previous worn study by determining the extent wearer DNA collected from the M-Vac® as opposed to a traditional collection method, such as a cutting or a swab, had for the collection of total human DNA and the electropherograms for these evidence samples. For the quantities of human DNA collected for biological fluids, the traditional method of cuttings appeared to collect more than the M-Vac®, whereas the collection for touch DNA was greater for the M-Vac® than the traditional swabbing method. As for the electropherograms, some of the profiles for both traditional methods and the M-Vac® yielded either partial- or full-mixture profiles between the evidence sample and the wearer DNA. In conducting this study, a more realistic scenario encountered in casework was understood, which is an important step in validating new instrumentation into a forensic DNA laboratory setting.

### M-Vac®, Sample Collection, Wearer DNA



**B74 Venturi Vacuum-Enhanced Collection and Recovery of Touch DNA Samples Coupled With Direct Polymerase Chain Reaction (PCR) for Forensic DNA Typing**

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**WITHDRAWN**

## B75 The Success Rate of Rapid DNA Technology in Genealogical Determination in Forensic Science

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**Learning Overview:** After attending this presentation, attendees will better understand the role of Rapid DNA technology in paternity studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling paternity determination studies to be carried out in a shorter time and with less contamination.

DNA analyses, which are routinely used for various purposes in basic sciences or medical sciences, are used in forensic sciences to provide objective evidence to courts. In forensic sciences, both the determination of paternity-kinship relations and identification of biological samples (blood, bloodstain, semen, semen stain, saliva, saliva stain, hair, bone, etc.) collected from the crime scene are performed by DNA analysis.

In paternity studies, by analyzing the biological samples taken from the mother, the suspect father, and the child; it is determined whether the suspected individual is the father of the child or not. In addition; biological fluids and tissues (blood, semen, saliva, hair, bone, etc.) or the stains of these, which were collected from the crime scene, are identified in the criminal investigations to determine who this evidences may belong to.<sup>1-3</sup>

Various commercial kits are available to enable easy obtaining of Short Tandem Repeat (STR) profiles. The process for obtaining profiles in routine studies is long and arduous. However, the time to reach the perpetrator is very important. Catching the perpetrator as soon as possible can prevent a second victimization in that process. For this reason, it is important to shorten and facilitate the analysis processes to obtain DNA profiles in order to catch the perpetrator quickly. In parallel with this, various studies have been carried out and are still being developed to facilitate the stages of obtaining and interpreting profiles.<sup>4</sup> The RapidHIT® System, which is developed in addition to the devices used routinely for this purpose, is a fully automatic DNA analysis device that enables the obtainment of STR profiles.<sup>2</sup> This system is a complex system that combines the routine laboratory steps by making isolation, DNA quantification, polymerase chain reaction, capillary electrophoresis, and data analysis all together to create DNA profiles in a short time.<sup>5-8</sup> In addition, the RapidHIT® system, which reduces the risk of contamination by keeping analyst number at a minimum and having a single-stepped conventional processes, obtains DNA profiles compatible with the Combined DNA Index System (CODIS) in as little as 90 minutes.<sup>5,7</sup>

Rapid DNA technology, which is known to have ability to work with reference samples and whose improvement process is still in progress, has been used since 2012. However, it has been noted in the literature that the use of rapid DNA technology can have disadvantages in solving cases, such as complex ancestry and paternity cases studied in forensic science.<sup>5-8</sup>

The aim of this study is to determine whether it is possible to get results more quickly in forensic science studies for the determination of paternity by comparing DNA profiles obtained by using conventional methods and DNA profiles obtained from fast DNA technology that gives results in as little as 90 minutes.

In this study; two buccal swab samples to study by using two different devices were taken from 90 volunteers in total, which were from 30 different families that consisted of mothers, fathers, and children. Twenty-four STR profiles of samples were obtained using the RapidHIT® device. When the profiles of 90 individuals from 30 families were examined by using the RapidHIT® System, a full profile was obtained in 81 individuals. In addition, a partial profile was seen in two individuals, while an allele drop was found in seven individuals. Routine DNA analysis was also used to study samples of the same individuals in steps that consisted first of DNA extraction and quantitation, second by amplification of 24 STRs in a thermal cycler, and third by separating STR loci in capillary electrophoresis. In the last step, the results of both systems were analyzed by cross comparison.

As a result of this study, the success rate of Rapid DNA technology, whose advantages were sorted according to routine DNA analysis in paternity testing, was revealed.

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### Forensic Genetics, Human Identification, Rapid DNA Technology



## **B76 GlobalFiler® IQC Amplification Kit Validation**

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**WITHDRAWN**

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## B77 The Identification of Drugs in Powder Form With No Sample Preparation Via Headspace Solid-Phase Microextraction (SPME) and Gas Chromatography/Mass Spectrometry (GC/MS) Methods

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**Learning Overview:** The goal of this presentation is to inform attendees of the use of high-temperature headspace SPME and GC/MS for the detection of various drugs, including methamphetamine, cocaine, and fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the use of high-temperature headspace SPME for the identification of drugs in powder form.

SPME is a technique in which analytes are sorbed onto a fiber coated with a polymeric material. SPME methods may be used in conjunction with GC/MS to identify illicit and licit drugs often within a matrix. These methods include headspace, immersion, and Total Vaporization (TV) SPME. When immersion and TV-SPME are utilized, a sample is dissolved into a matrix, such as methanol or acetonitrile; however, illicit drugs are often found in the form of powders and in a mixture with other drugs or adulterants. For this work, traditional headspace SPME methods were utilized for the detection of various drugs in powder form using a Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) SPME fiber.

It was hypothesized that headspace SPME could be used to identify drugs in powder form by placing the drug into a headspace vial and heating the vial to 120 °C. Drugs of interest included methamphetamine, pseudoephedrine, caffeine, cocaine, procaine, inositol, heroin, diphenhydramine, fentanyl, and pharmaceutical tablets, including hydrocodone and oxycodone. These drugs were analyzed individually as well as in a realistic mixture. Seized drug samples were also analyzed. To analyze these drugs using a headspace SPME method, a sample of the powdered drug or drug mixture (~1–2mg) was placed into a headspace vial with no prior sample preparation or extraction. This vial was then heated to 120°C inside of a Gerstel agitator and the sample was adsorbed onto the SPME fiber for ten minutes. If derivatization was necessary, the PDMS/DVB fiber was first inserted into a vial containing the appropriate derivatization agent, then inserted into the sample vial for 30 minutes. All drugs, excluding inositol, were identified in powder form using this headspace SPME method without any prior sample preparation and without dissolving the drug in a solvent. These drugs were successfully identified individually as well as within a realistic mixture. This headspace SPME method is simple, efficient, and cost effective for the detection of legal and illegal drugs.

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**Drugs, Solid-Phase Microextraction, GC/MS**

## B78 The Effects of Degradative Stress on Vapor Analysis of Fentanyl

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**Learning Overview:** After attending this presentation, attendees will better understand how degradative stress affects the compounds found in the headspace profile of fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the origins of the compounds found in the unique headspace profile of fentanyl.

Fentanyl is a Schedule II synthetic opioid with a potency 100 times greater than that of morphine. It was first synthesized in 1960 by Dr. Paul Janssen and is used to treat severe short-term and chronic pain in cancer patients or as an anesthetic.<sup>1,2</sup> According to the Centers for Disease Control (CDC), in 2017, there were 70,000 overdose victims in the United States, 30,000 of which were attributed to fentanyl or a related analog, a 45% increase of fentanyl overdoses from 2013.<sup>2</sup> However, with recent restrictions put in place on fentanyl prescriptions, most of the fentanyl abused is purchased illegally. Illegal fentanyl is produced mainly in China and enters the United States via criminal transport organizations from places such as the Caribbean and Mexico. With an increase in the overdose rate, as well as the constant flow of fentanyl into the country, it is important for law enforcement to have a reliable method to detect fentanyl at ports of entry. Furthermore, it is even more pressing for this method to be non-contact to maintain the safety of first responders and other law- and customs-enforcement agents.

Vapor detection, such as by canine or electronic nose, has great potential as a non-contact detection method. Understanding a substance's unique headspace profile is imperative for the development of any vapor detection. For instance, knowledge of the headspace profile can also be utilized to develop safe training aids for canine detectors. This research focused on determination of the origin of the compounds making up the headspace profile of fentanyl. This presentation will describe the use of degradative stress to determine the origin of each compound in the headspace profile of fentanyl.

Solid Phase Microextraction (SPME) followed by analysis using Gas Chromatography/Mass Spectrometry (GC/MS) were used to determine the Volatile Organic Compounds (VOCs) that compose the headspace profile of fentanyl. In order to determine the origin of the VOCs in fentanyl's headspace profile, a series of degradative experiments were performed. Fentanyl samples were subjected to degradation via thermal and oxidative stress. For thermal degradation, the headspace profile was studied at 40°C and ambient (22°C) under dry and humidified conditions. For oxidative degradation, fentanyl samples were placed in either an inert or oxygen-rich environment at either room temperature or 40°C.

Based on these studies, a number of analytes were identified in the headspace profile of fentanyl. These compounds included styrene, benzaldehyde, aniline, N-phenylpropanamide, and N-Phenethyl-4-Piperidone (NPP). Out of these compounds, benzaldehyde was indicative of oxidative degradation. The identification of these VOCs in the headspace of fentanyl and fentanyl undergoing degradation provided essential information of fentanyl's unique headspace signature as well as to the origin of these compounds.

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### Fentanyl, SPME-GC/MS, VOCs



## **B79 Nuclear Magnetic Resonance (NMR) Spectroscopy as a Tool for the Quantitative Determination of Cannabinoids and Related Compounds in Sugary Beverages**

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**WITHDRAWN**



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## B80 The Differentiation of Synthetic Cathinone Isomers by Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) and Multivariate Analysis

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**Learning Overview:** After attending this presentation, attendees will have learned about the differentiation of synthetic cathinone isomers using GC/EI/MS and multivariate analysis. Attendees will learn about the concepts of multivariate discriminant analysis, characteristic ion ratios, and methodologies to reduce the required number of replicate sample injections for multivariate classification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners with a multivariate analysis approach for the differentiation of synthetic cathinone isomers using GC/EI/MS. Specifically, the development of methodologies to decrease the required number of replicate sample injections for multivariate classification will enhance the applicability of this approach to forensic practitioners.

The central hypothesis of this study is that each synthetic cathinone isomer has a unique EI fragmentation pattern that allows for the identification of characteristic ion ratios and, ultimately, the successful identification of each isomer using multivariate classification. Additionally, it is hypothesized that the characteristic ion ratios used for synthetic cathinone isomer differentiation will remain conserved across various sample concentrations.

An Agilent® GC/EI/MS was used for the analysis of synthetic cathinone constitutional and positional isomer drug standards. The synthetic cathinone constitutional isomer drug standards included dibutylone, eutylone, and pentylone, whereas the synthetic cathinone positional isomer drug standards were comprised of 2-, 3-, 4-chloroethcathinone, and 2-, 3-, 4-methoxymethcathinone. Each synthetic cathinone drug standard was analyzed at concentrations of 10ppm, 100ppm, and 1,000ppm to assess the influence of concentration on the observed characteristic ion ratios. For each synthetic cathinone drug standard, the relative ion abundances were extracted for further data analysis.

Statistical Package for the Social Sciences (SPSS) software from the IBM® Corporation was used to perform Canonical Discriminant Analysis (CDA) for each set of constitutional and positional isomers. CDA is a supervised multivariate discriminant analysis technique capable of discriminating between two or more groups by maximizing the between-group variance and minimizing the within-group variance. In addition, structure matrix correlation data was used to identify characteristic ion ratios that allowed for the differentiation of constitutional or positional isomers. Finally, the use of multiple mass spectral scans across the chromatographic peak and reducing the number of extracted ions used to create the CDA models were evaluated as methodologies to reduce the required number of replicate sample injections for multivariate classification.

The results indicate that CDA models can be used to differentiate between synthetic cathinone constitutional and positional isomers based on characteristic ion ratios derived from subtle differences in EI fragmentation patterns. Likewise, the use of multiple scans across the chromatographic peak and the development of CDA models based on a reduced number of extracted ions show promise as methodologies to reduce the number of required replicate sample injections for multivariate classification.

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### Seized Drugs, EI Fragmentation, Multivariate Analysis

## B81 The Development of Colorimetric Methods for Marijuana Identification Via Mobile Phone Imaging

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**Learning Overview:** The goal of this presentation is to display the methods developed that allow for a mobile phone calibration of common field tests historically used to identify marijuana.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by laying the groundwork for a colorimetric field test used to quantitate the amount of Tetrahydrocannabinol (THC) in suspected marijuana samples.

Marijuana has been at the forefront of social and political dialogue with respect to medical and recreational use. Congress recently passed the “Hemp Farming Act of 2018,” which allowed for the cultivation and sale of hemp. This law defines hemp as any part from the plant *Cannabis sativa* L. containing less than 0.3%  $\Delta$ -9-Tetrahydrocannabinol (THC) by dry weight. Because of the precision of this definition, some level of quantitative test is now necessary to make the distinction between hemp and marijuana in field settings. In response, some law enforcement organizations are now using the 4-Aminophenol (4-AP) test, which has been claimed to draw this distinction based on different color responses to differing ratios of THC and Cannabidiol (CBD). However, validation studies of the 4-AP test have demonstrated its inadequacy for the purposes of quantitatively identifying THC levels. There remains no quantitative field test in circulation. This issue further complicates the legality of seizures with respect to reasonable suspicion.

Due to recent advances in photographic capability, cell phones are widely viewed as tools capable of objective field testing. Recent studies have also demonstrated the use of various pixel analyses to achieve quantitative results using colorimetric tests. Toward the goal of differentiating hemp and marijuana, this study proposes the use of two well-known presumptive tests (i.e., the Duquenois-Levine and Beam tests) in conjunction with cell phone photography. Tests were performed using solutions of THC and CBD standards allowing for external calibration of both the Duquenois-Levine and Beam Tests. Duquenois-Levine is a field test invented on the early 1900s and is a three-step mixture with plant extract, the first being acetaldehyde, vanillin, and ethanol, the second simply concentrated hydrochloric acid, and the third being a chloroform extraction of the chromophore produced by the first two steps. The Beam test is as well a test made in the early 1900s but has found less popularity in law enforcement because it reportedly does not react with THC. This test consists of the addition of 20% potassium hydroxide weight by volume in ethanol to the plant extract. As anticipated, issues with lighting and camera settings contributed to substantial hurdles in data acquisition from these images. Changes in the approach to data analysis and alterations to the sample medium to provide a more homogenous image can potentially mitigate these problems. Further development of these methodologies is expected to lay the foundations for a reliable field test to differentiate hemp and marijuana.

### Drug Chemistry, Marijuana, Field Test

## **B82 The Power of Knowing Now: Rapid Drug Screening Using Atmospheric Solids Analysis Probe/Mass Spectrometry (ASAP/MS)**

*Michelle Wood, PhD\*, Waters Corporation, Wilmslow, Cheshire SK9 4AX, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will understand the technique of ASAP/MS. Discussion will include a presentation of alternative ionization mechanisms together with demonstration of how collision-induced dissociation can improve the specificity of drug identification. Principles will be illustrated with data for common drug substances. Attendees will gain an appreciation of the simple workflow and, importantly, understand the performance of this alternate screen with seized drug samples. The findings for a series of drugs collected by the authorities from music events will also provide insight into drugs used in this particular cohort.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how an alternative rapid drug screen can improve laboratory efficiency and reduce sample backlog.

The increase in number, diversity, and potential toxicity of drugs is a major concern; it also presents significant challenges for the forensic laboratories that are involved in the analysis of seized substances and are under pressure to produce results quickly. Most forensic drug chemistry laboratories follow Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations that stipulate that two independent techniques should be used to analyze a drug sample. Typical workflows may include colorimetric or Thin-Layer Chromatography (TLC) presumptive analysis followed up by a more selective method such as Gas Chromatography/Mass Spectrometry (GC/MS). However, for many drugs, colorimetric tests are not be available or result in a high rate of false positives and TLC analysis can be time-consuming. This can lead to too many samples requiring analysis by GC/MS leading to sample bottlenecks and sample backlogs. Consequently, methods that can facilitate a fast, but accurate, screening of drugs are of interest.

The aim of this study was to evaluate the performance of RADIANT-ASAP, a compact device based on ASAP/MS for rapid drug screening and to compare data with an established screening method based on High-Resolution Mass Spectrometry (HRMS).

Samples (certified reference material, pills, powders, resin) were analyzed following a simple dilution with methanol and subsequent “dipping” of a glass capillary into the sample. Following insertion of the capillary into the device, mass detection was performed using full scan MS  $m/z$  60650. The ASAP ionization process is similar to atmospheric pressure chemical ionization, whereby a heated desolvation gas is used to volatilize the sample and a corona discharge to ionize; this typically results in protonation for most polar drugs (i.e.,  $M+H^+$ ). To further enhance specificity, the analysis was acquired simultaneously at four differing cone voltages (i.e., 15, 25, 35 and 50V), which resulted in the generation of both precursor and product ions. Data was processed by LiveID™ software that provided real-time matching of acquired data to a spectral library and calculated an average match factor.

The study assessed various samples, including 40 Certified Reference Material (CRM), 20 pharmaceuticals, 10 natural supplements/herbal medications, and more than 60 unknown samples that had been confiscated at various music events/venues by the local police. A small library was generated from ASAP/MS of the CRM. Analysis of the other preparations (e.g., pharmaceutical and seized samples) demonstrated very good qualitative agreement when compared with this library. For the samples acquired from music events, ASAP/MS indicated that 40% of the samples contained ketamine, 30% contained MDMA, and 20% contained cocaine. Other drugs identified included mixtures of these three drugs, MDMA/MDEA, novel psychoactive substances, sildenafil, etc. Confirmatory analysis by HRMS showed excellent agreement (> 95%) with the major components identified by ASAP/MS.

The technique is quick, easy-to-use, and is very promising for a rapid identification of drug substances. Results were obtained within two minutes and showed very good qualitative agreement with the comprehensive HRMS screening method.

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### **Direct MS, Rapid Drug Screening, Seized Drug Analysis**



## **B83 Method Development for Separation and Quantitation of 17 Cannabinoids Using Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

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**Learning Overview:** After attending this presentation, attendees will learn about the different methods used to quantitate cannabinoids using GC/MS and LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into GC/MS and LC/MS/MS method development focused on the separation of cannabinoids for quantitation.

Cannabis has been considered a Schedule I controlled substance since the 1970s. Federal, state, and local forensic labs have traditionally used qualitative methods to identify the presence or absence of Tetrahydrocannabinol (THC) in confiscated cannabis samples. However, the 2018 Farm Bill removed hemp from the controlled substances list and defined it as cannabis containing less than 0.3% total THC content. By defining a concentration threshold, the 2018 Farm Bill created a need for THC quantitation and, with that, an inherent requirement for quantitative methods. A cannabis research program has been established to assist the forensic community in making quantitative measurements in various cannabis samples through the development of analytical methods, a quality assurance program, and cannabis reference materials. The work presented will include LC/MS/MS and GC/MS method development for the quantitation of 17 cannabinoids in multiple cannabis materials.

The LC/MS/MS method development utilized MS/MS selectivity to reduce necessity of LC separation of cannabinoids with distinct ion transitions, allowing for focused chromatographic separation of the isomeric cannabinoids. To determine the most effective chemistry for cannabinoid separation, reversed phase C18 and biphenyl columns were evaluated. Separation was achieved using gradient elution, with 0.1 % formic acid in water and 0.1 % formic acid in methanol as mobile phases. Data acquisition was carried out in multiple reaction monitoring mode, using positive electrospray ionization for the neutral cannabinoids and negative electrospray ionization for the acidic cannabinoids.

The GC/MS method described here focuses on the baseline separation of nine neutral cannabinoids while limiting the total run time to less than ten minutes, permitting a higher sample throughput in forensic laboratories. As part of the method development, a detailed comparison of GC column stationary phase and oven temperatures were evaluated with an emphasis placed on optimizing separation and sensitivity for delta-9-THC. One of the GC stationary phases evaluated included the non-polar 5% phenyl phase that is currently utilized in forensic laboratories for measuring delta-9-THC in cannabis samples. However, a second stationary phase consisting of 35% phenyl was found to provide a superior separation. Additionally, the initial oven temperature was optimized to identify the highest temperature that provided the earliest elution of the cannabinoids without sacrificing chromatographic separation. As a result, a quick ten-minute quantitative method using a 35% phenyl stationary phase was developed and evaluated for the analysis of various cannabis plant and oil samples.

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### **Chromatography, Cannabis, Mass Spectrometry**

## B84 The Detection of Chloroquine in Opium Using Gas Chromatography/Mass Spectrometry (GC/MS): A Toxic Adulterant

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**Learning Overview:** The goal of this presentation is to establish the fact that chloroquine detection in higher concentrations in routine opium samples is of major concern for opium ingesters/traffickers, where the chances of toxicity coupled with opium alkaloids are manifold. Furthermore, chloroquine may also be characterized as a major marker of opium resin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding the presence of chloroquine in opium resin specifically related to opium ingesters/abusers and its toxicity along with opium alkaloids. Moreover, this presentation will provide information that will help to determine the source of opium as well.

Opium is a dried milky exudate or latex obtained from unripe pods of *Papaver somniferum* L. Opium poppy contains more than 80 tetrahydrobenzyl isoquinoline-derived alkaloids including morphine, codeine, papaverine, noscapine, laudanine, and laudanosine. Various analytical methods are applied for qualitative detection and quantitative estimation of opium alkaloids. The opium is usually smuggled as “body packers,” “swallowers,” or “mules” using plastic bags, balloons, or condoms. Chloroquine is an antimalarial drug of the 4-aminoquinoline group. The lethal effects of chloroquine intoxication such as congestive heart failure and myopathy leading to complete heart block are observed in suicide or murder cases. It is observed as adulterant in heroin in Afghanistan, Iran, and Pakistan along with acetaminophen, phenolphthalein, and caffeine. It is quite cheap and easily available due to its crystalline nature. The addition of chloroquine in heroin samples is debatable as some Malaysian experts suggested that it increases the heroin efficacy by inhibiting the P-450 cytochrome.

The case samples submitted for forensic drug analysis were randomly selected (2017, 2018, and 2019) for analysis of opium. The methanolic extract of opium resin and plant material was analyzed using GC/MS (an Agilent® GC system 7890A series coupled to MSD 5977 with ALS 7693). The following parameters were used for GC/MS analysis.

<b>GC (7890B)</b>	
Inlet	Split mode (50:1)
Inlet temperature	250°C
Inlet pressure	13.332psi
Oven program	150°C for 1min then 25°C/min to 300°C for 5min with total run time of 12min.
Carrier gas	Helium (flow rate 1mL/min) with injection volume of 1µL.
Column	DB-5MS column 30m×250µm×0.25µm (Length x Internal Diameter x Film thickness)
<b>MS (5975C) in Scan Mode</b>	
MS Source	230°C
Scan Range Mass	43–550
Quad temperature	150°C

Major ion peaks for chloroquine were compared with that of the reference literature. Results were also compared with authentic reference library.

Morphine, codeine, thebain, papavarine, and noscapine were detected in both opium resin and plant material. Chloroquine was detected in large abundance in the majority of opium resin samples while no traces of it was detected in crushed plant parts.

This detection of higher quantity of chloroquine in opium resin enhances toxicity alone or in combination. The intoxication of chloroquine leading to suicidal effects has been vastly studied and found to be lethal at higher doses, causing a threat to the opium ingesters and body packers.

### Forensic Analysis, Opium, Chloroquine

## B85 An Electrochemical Analysis of Fentanyl and Fentalogs Toward a Rapid Screening Assay

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**Learning Overview:** After attending this presentation, attendees will have information on an electrochemical technique to analyze fentanyl, as well as a stronger understanding of the electrochemistry of fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating exploration into alternative methods to detect fentanyl using electrochemistry and how that could facilitate the screening of fentanyl analogs. This presentation will also showcase another example of how electrochemistry can be used as an analytical technique in forensic investigative sciences.

The use of opioids is on the rise nationwide, and the potential danger of an unintentional exposure is increasing. One of the current deadliest synthetic opioids is fentanyl.<sup>1</sup> There are also numerous fentanyl analogs that have been synthesized, often illicitly, that are hazardous, especially as some of them are more potent than fentanyl itself.<sup>2</sup> There are many laboratory-based methods used to detect fentanyl, but they lack the portability and rapid response necessary for early detection in the field. One of the major issues with detecting fentanyl analogs is their number and the difficulty to characterize them and establish standardized methods as fast as new illicit analogs are encountered in street samples.<sup>2</sup>

The focus of this project is to use electrochemistry as a fast detection method answering those needs and allowing for early detection of fentanyl and fentanyl analogs. In the body, fentanyl undergoes an oxidative N-dealkylation reaction which generates metabolites.<sup>3</sup> The electrochemical reaction of fentanyl appears to be mimicking this process, with an irreversible oxidation, generating metabolites in solution, which can also be observed by electrochemistry.<sup>4</sup> It is hypothesized that this N-dealkylation would be common to fentanyl and its analogs, thus generating a standard method for the fentalogs. Different electrochemical techniques are being utilized and optimized to detect fentanyl, including cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry. Using these techniques, the irreversible oxidation peak of fentanyl is being monitored. The intensity of the oxidation decreases over subsequent scans, concomitant to the development of additional peaks for the metabolites. Fentanyl and fentanyl metabolite peaks are visible in multiple supporting electrolytes such as sodium chloride, potassium chloride, and Phosphate Buffer Saline (PBS). The conditions to analyze fentanyl such as concentration of supporting electrolyte, potential range, and pH are explored and optimized. The long-term goal of this project is to develop a method common to fentanyl and fentanyl analogs to allow for a rapid detection of these drugs in one test that will benefit in-field techniques.

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### Fentanyl, Electroanalytical Chemistry, Fentanyl Analogs

## B86 Effective Field Analysis of Low-Dose Fentanyl Mixtures by Portable Vibrational Spectroscopy and Portable Gas Chromatography/Mass Spectrometry (GC/MS)

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**Learning Overview:** After attending this presentation, attendees will understand how portable Infrared (IR) spectroscopy and GC/MS can be used in the field to accurately identify fentanyl in low-dose tablets.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and law enforcement communities by providing new information regarding the on-scene analysis of low-dose illicit fentanyl tablets by portable instrumentation.

Fentanyl is a potent, synthetic opioid that has caused tens of thousands of deaths since 2013, the start of the latest opioid epidemic.<sup>1</sup> It has been used historically as a powerful pain reliever but recently has become extensively abused. Fentanyl has been found in combination with several other drugs of abuse such as heroin and cocaine, frequently pressed into tablets, whether on its own or in conjunction with other active ingredients and additives. The availability of fentanyl in street samples poses a risk not only to users who may not be aware that their drug of choice may now also contain fentanyl, but also to law enforcement personnel who are responding to scenes of illicit manufacture, selling, or overdoses. When encountering fentanyl that is not legally manufactured, the amount of fentanyl present in the tablet is unknown and potentially fatal.

The availability of fentanyl in pressed tablets poses a unique issue. The fentanyl that is present in these tablets is commonly a very low amount (~1%) and therefore not easily identified by traditional field means. Because traditional field-testing methods routinely deployed may not be able to detect such low amounts of fentanyl relative to other filler ingredients, there has arisen a need to develop more effective methods of analysis.

This research was conducted by making and analyzing mixtures of various percent compositions of fentanyl in additives. Mixtures ranged from 50% active ingredient to 1% active ingredient of fentanyl in combination each with acetaminophen, lactose, and mannitol. A mannitol:fentanyl:heroin mixture was also made in ratios of 65:10:25 and 32.5:5:12.5. Each mixture was analyzed as a solid in triplicate on a next generation portable IR spectrometer equipped with a diamond Internal Reflection Element (IRE) for Attenuated Total Reflection (ATR) analysis. This instrument is capable of achieving lower detection limits than other available portable IR instruments because of higher throughput optics and targeted library-search algorithms. Results showed that fentanyl was identifiable down to 1% (w/w) in both mannitol and lactose using this new portable IR spectrometer. For the mixture in acetaminophen, the major fentanyl peaks overlap with those of the acetaminophen, which prevent detection at low levels, thus extractions were required. Two base-extraction protocols were evaluated using the 1% mixtures of fentanyl: (1) 1M sodium hydroxide and chloroform, and (2) 1M sodium hydroxide and hexane. The organic layer containing fentanyl was directly analyzed by putting a drop or two of the extract onto the IRE, then allowing the solvent to evaporate. Both of these extraction methods were successfully able to remove all detectable acetaminophen and enable easy fentanyl identification from these low dose mixtures.

Portable GC/MS is emerging as a valuable tool for the on-scene detection and identification of illicit drugs. However, low-dose tablets present a challenge for these instruments because the high quantity of adulterant (in relation to the quantity of fentanyl) saturates the MS, which then requires significant maintenance and cleaning. Thus, the fentanyl extracts were also analyzed using a portable GC/MS equipped with simplified sampling. The portable GC/MS positively identified fentanyl in extracts made from both previously described methods.

This research demonstrated that portable IR spectroscopy and GC/MS are valuable tools for law enforcement and other first responders for the field identification of low-dose fentanyl tablets.

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### Fentanyl, Field Analysis, Portable Instrumentation

## B87 The Roles of Projectile Energy and Apparel Fabric Damage in Assessing the Magnitude of Long-Range Shooter Distances

*Nicholas Gogola, BS\*, Hamden, CT 06514; Peter R. Valentin, MSFS, University of New Haven, West Haven, CT 06516*

**Learning Overview:** The goal of this presentation is to show how the relationship between projectile energy and fiber end morphology can be used to assess muzzle-to-target distances in long-range shooting incidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a link between projectile energy and apparel fabric damage and by demonstrating the value in microscopic observation of fiber end morphology for forensic determination of long-range shooter distances.

In most short-range shootings, muzzle-to-target distance is determined via Gunshot Residue (GSR) using colorimetric assays such as the sodium rhodizonate or Modified Griess test or by scanning electron microscope to detect lead, barium, and antimony particles. However, at distances where little to no GSR is found on evidentiary items, these methods become unsuitable. In many such firearms-related homicides, the projectile perforates one or more articles of the victim's clothing. Through careful examinations of these bullet-perforated fabrics, critical information regarding the shooter's distance can be uncovered.

Observed morphologies of the fabric, yarns, and fibers of apparel textiles are commonly used to determine the type of weapon used in the commission of a crime. These include changes to yarn spacing at the fabric level, degree of fraying or melting of the yarn ends at the yarn level, and pushing, shearing, tearing, or cutting of individual fibers at the fiber level. When the damage has been found to be caused by a bullet, those same observations can be utilized to approximate the bullet's energy. Morphological factors at these three levels will vary distinctly with input energy. Bullet holes will show less uniformity, yarns will show an increased degree of fray, and individual fibers will show a smaller magnitude in shearing with increased tearing and pushing of fibers when projectile energy has been significantly decreased.

The longer a projectile travels through the air, the more it will be slowed by air drag. The kinetic energy of a projectile, proportional to its velocity squared, therefore continues to decrease the longer the projectile is in flight. Long-range projectiles thus will have lower energy levels at the target than their short-range counterparts. Furthermore, the terminal velocity, along with manufacturer data such as muzzle velocity, can mathematically determine an approximate muzzle-to-target distance.

The hypothesis of this research rests on these two axioms: (1) as input projectile energy decreases past a threshold energy level, the observed fabric and fiber damage will show distinct morphologies, and (2) projectile energy loss can approximate the long-range distance of the shooter. If both of these axioms hold true, then there must exist one or more threshold distances beyond which the observed morphologies of bullet holes in apparel fabrics are distinguishable from those within that distance. This will create an opportunity for approximated long-range shooting reconstruction to feature prominently in casework.

Various shooter distances were simulated at an indoor range. Shots were fired at swatches of seven different fabrics from distances of 1m, 3m, 5m, and 10m. A fraction of the gunpowder in the cartridges was removed at 5m and 10m in an attempt to decrease the muzzle velocity, which decreased the energy imparted to the fabrics. This decreased energy transfer simulates the projectile having traveled further than 10m and decelerating more due to air drag. Downloaded cartridges contained 90%, 75%, and 60% by mass of the manufactured gunpowder inside the cartridge. The seven fabrics consisted of a cotton t-shirt, cotton jeans, polyester blouse, polyester athletic shorts, cotton/polyester blended t-shirt, men's button-down shirt, and sweatshirt materials. Thickness and weave-pattern construction were varied among the sample population as well. Stereo microscopy was employed to study fabric and yarn level damage, and polarized light microscopy was employed to study fiber level damage.

By understanding the link between projectile energy at the time of fabric perforation and observed morphologies in apparel fabrics and fibers, the forensic scientist can determine for investigators whether the shooter was near or far from the victim when the shot was fired, which can aid in scene reconstruction as well as evaluation of conflicting accounts.

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### Fibers, Fabric, Projectile Energy



## B88 Forensic Textile Dye Analysis by Thermal Desorption Direct Analysis in Real-Time Mass Spectrometry (DART<sup>®</sup>-MS) and Raman Microscopy

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**Learning Overview:** After attending this presentation, attendees will better understand how DART<sup>®</sup>-MS and Raman microscopy can be used for forensic textile dye analysis. The DART<sup>®</sup>-MS and Raman methods can be used to distinguish among different types of dyes and provide characteristic information for forensic fiber identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by comparing the two advanced techniques, DART<sup>®</sup>-MS and Raman microscopy, for their application on forensic fiber and textile dye analysis, which will hold great potential across a broad range of topics in the analytical chemistry area and will impact the forensic science community, especially the area of forensic fiber analysis.

The Thermal Desorption(TD) -DART<sup>®</sup>-MS method was developed for the analysis of an array of blue dyes in this study. Past experimentation has shown that the TD-DART<sup>®</sup>-MS can be used to identify the polymeric backbone structures, and obtain the chemical information, of common textile materials such as cotton, nylon, polyester, cellulose triacetate, poly(propylene), and poly(acrylonitrile).<sup>1</sup> Currently, there are thousands of textile dyes derived from natural sources or made synthetically, and these are classified into different categories according to their application method and chemical composition. The ability to correctly identify an unknown fiber is of great forensic importance, and this evidence is often used to place a suspect's carpet or clothing at a scene.<sup>2-3</sup>

This study was composed of nine blue dyes from four categories (acid, basic, reactive, and vat blues) that are commonly used in manufactured textiles. A TD-DART<sup>®</sup>-MS method was developed to analyze the dyes, which were applied on an array of common fibers, including silk, cotton, and nylon. The mass spectra were collected, and the multidimensional profiles, including both the physical properties of melting point and chemical information, were analyzed and compared to each other in order to locate identifiable signals. The signals of the dyes were distinguishable from their fiber, and the characteristic ions were unique for each dye structure. The DART<sup>®</sup>-MS results were compared to those obtained by Raman microscopy with and without Surface-Enhanced Raman Scattering (SERS) by colloidal silver nanoparticles. The benefit of SERS is to limit the excitation depth of the Raman signal from the fibers so that signal due to the dye is enhanced relative to the underlying polymeric fibers. The Raman microscope offers molecular mapping capability that combines the morphological and chemical information of fibers for unequivocal identification fiber origins. The study found that the TD-DART<sup>®</sup>-MS method requires a single fiber sample size and a sample preparation time of approximately two minutes, but it offers a multidimensional profile within approximately seven minutes. Due to this method being both simple to perform and having high sample throughput potential, this method may significantly contribute to the identification of unknown dyes on fibers in comparison to the traditional, slower methods that require the extraction of dyes or chromatographic separation.

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### Textile Dye, DART<sup>®</sup>-MS, Raman Microscopy

## **B89 The Effects of Sample Preparation Optimization on Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS)**

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**Learning Overview:** The goal of this presentation is to demonstrate how optimization of sample preparation and particle dispersal parameters impact soil mineral analysis by PCRS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an optimized soil mineral sample preparation and particle dispersal method as a part of the development of an analytical approach to forensic soil analysis using PCRS.

Soils consist of minerals, organic matter, microorganisms, anthropogenic, and other materials. Each of these constituents within the soil matrix can provide a significant amount of information for forensic soil analysis. Unfortunately, there has been a decrease in forensic soil examinations due to a plethora of issues, including the perceived complexity of soil analysis without accompanying statistical significance determinations, the time required for traditional microscopic analysis, budget reductions for trace examinations, and the failure of crime scene investigators to recognize the value of soil evidence. PCRS and Morphologically Directed Raman Spectroscopy (MDRS) are novel analytical techniques that have the potential to automate soil analysis methods. They provide reliable information on particle size distribution and particle morphology (circularity, elongation, etc.) and subsequently perform Raman spectroscopic analysis of isolated individual particles to accurately identify soil mineralogy.

Sample preparation is a key aspect to any microscopic investigation. Measurements are performed on individual microscopic particles, such as soil mineral grains. For meaningful results, and to maximize the evidentiary value of soil samples being analyzed, sample preparation needs to be optimized. It is important to ensure particles are reproducibly, evenly, and wholly distributed onto a substrate for analysis, and therefore the dispersion parameters must be optimized.

PCRS uses computer-controlled vacuum-type powder dispersion while MDRS employs a positive pressure dispersion technique. These dispersion systems are used to create a uniform distribution of microscopic mineral particles with good spatial separation. Poor dispersion may lead to clustering, overlapping, or fractured particles, which negatively affect the automated analysis of PCRS. Therefore, having optimized dispersion parameters allows accurate collection of data from individual particles, thus maximizing the information obtained from the soil samples and increasing the discriminating power of PCRS.

This study specifically considers the dispersion using PCRS in detail, though many of the elements are valid for both. Nine soil samples of varied geological provenance (three sand, three loam, and three silt) were collected from around the northeast United States. Each sample was air dried to a powder and subsequently coned and quartered. For each sample, one-quarter was analyzed without further processing, one-quarter was washed and dried before analysis, one-quarter was washed, dried, and sieved before analysis, and one-quarter was retained for traditional forensic soil analysis. Soil samples were then dispersed onto a glass slide or plate by computer-controlled vacuum-type powder dispersion. The variables assessed in this research included washing of the samples, sieving of the samples, and optimizing vacuum dispersion parameters (sample volume, vacuum pressure, diameter of dispersal element, and time for dispersal and settling). Dispersion was assessed microscopically via PCRS for reproducibility, uniformity, dispersion density, and the maintenance of particle morphological characteristics throughout the processing. Determination of the optimal sample preparation parameters is a critical first step in creating a reliable method for obtaining the most forensically relevant information from soil evidence using PCRS.

### **Raman Spectroscopy, Minerals, Vacuum Dispersion**

## B90 An Evaluation of Score-Based Likelihood Ratios (SLRs) for Glass Data

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**Learning Overview:** The goal of this presentation is to illustrate the use of statistical simulation to study the behavior of SLRs. Using realistically simulated data of trace elemental analysis for glass, this presentation compares the behavior of different SLRs to their likelihood ratio counterpart under the common source scenario and assesses the dependence structure that may arise due to the choice of a training sample.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a review of tools for assessing SLRs and dependence structure that may arise in their use.

The use of likelihood ratios is advocated as a way to provide a numeric assessment of the evidential strength during the forensic expert testimony. The development of such ratios requires the construction of probability models that might be challenging, if not infeasible, to estimate. To avoid this, researchers can implement similarity scores as a way of reducing the complexity of a model into a potentially lower-dimensional metric, but there is still concern regarding behavior of such a score-based likelihood ratio.<sup>1,2</sup>

Consider the following scenario where a forensic examiner evaluates two glass fragments. We can frame this problem under the common source problem as follows: given two fragments (X and Y) that come from their respective windows (W1 and W2), examiners will deal with the hypothesis that windows one and two are the same, meaning that the fragments come from the same source, or the alternative hypothesis that they are different.

Without relying on normality assumptions, the researcher may approach the problem by computing a similarity score, a quantitative measure of how similar the chemical compositions of two fragments are. Previous projects have proposed using a random forest similarity score, while others that study chemical composition have used distance-based scores when addressing chemical composition.<sup>3-5</sup> This score gives the examiner a quantitative input. Still, a reference is required to know if the score found is more likely under the same source or the different source hypothesis.

The approach described requires two key components: (1) the development of a similarity score, and (2) estimating the distribution of such scores under the same source or different source scenarios. For the second component, the researcher can construct a database of pairwise comparison where the ground truth is known, meaning if the pair is a known match (same source) or a known non-match (different source).

Inquiry regarding the behavior of SLR has been previously made in the literature.<sup>2,6</sup> A factor less explored is the dependence on the training database. To assess this, this study created realistically simulated data using the chemical composition of glass data.<sup>7,8</sup>

Using an approach inspired by machine learning, three datasets were simulated that play the role of training, testing, and validation sets to compare four score-based likelihood ratios: (1) Euclidean distance, (2) city distance; (3) random forest score, and (4) a random forest with an additional down sampling step during the training stage.<sup>9</sup>

In each simulation, this study used its designated training set to train the random forest model, the test set to estimate the distributions under both hypotheses. Lastly, this study used the validation set to illustrate two critical aspects. First, since the data was generated following a known model, this study compared the decision reached using the SLR and the corresponding LR in terms of the rate of misleading evidence and if they fall in the same range of strength of evidence. Secondly, using permutation of the simulated training, testing, and validation sets, this study presents measures of dependence on the training data.

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### Score Likelihood Ratios, Learning Algorithms, Glass Evidence

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## B91 Newly Commercialized Recycled Paints: Are They as Variable as They Seem?

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**Learning Overview:** The goal of this presentation is to share the knowledge acquired on the analysis of recycled paints. The few studies conducted to date show that the expected variation in composition is counterintuitive at first but makes much more sense if we consider the multiple numbers and origins of the paint sources.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting attendees with new information on recycled paints. This presentation will help attendees make informed decisions when confronted with association/comparison cases by considering the particularities of this newly encountered type of paint trace.

A few recent studies have tried to evaluate the utility of differentiating paints at a production batch level with very mixed results, depending largely on the paint type.<sup>1-3</sup> A survey of the literature published to date shows that batch level differentiation is much more arbitrary and subject to many unknowns, such as the quality controls and adjustments the paints are undergoing, the varying origins of raw materials, or the decision to make changes in the paint recipes for improvement or discontinuity of raw materials.<sup>1,2</sup> All these reasons suggest that a particular production batch may suddenly present a substantially different composition than the one produced immediately before or after it. While forensic scientists are still undergoing studies to understand the potential of discrimination between paints, industrials have taken it a step further. Several paint companies now include a range of recycled paints among their proposed products. These recycled paints are composed of wastes collected by recycling plants. First sorted by their color and binder type (i.e., latex, alkyds), all the leftovers are mixed together in large tanks to form the basis material for future formulations.<sup>2</sup> This lot can be used as-is for lower quality paints or undercoats or may be adjusted to meet required specifications. Quality control for these recycled batches is obviously less precise, and a higher variation is expected in the esthetic properties of the paint. While it is not directly monitored by the producers, the variation in chemical composition should follow the same trends, if not greater.

A collaboration with a North American company allowed access to their samples, paint formulations recipes, and a summary of the quality controls they performed on each production batch. The whole study was conducted “blindfolded” and a final verification was made with the manufacturer to evaluate the accuracy of the results. The products obtained from the company consisted of white and colored recycled paint samples collected from different production batches. All samples were analyzed by microscopy, infrared spectroscopy, Raman spectroscopy and pyrolysis Gas Chromatography/Mass Spectrometry (GC/MS).

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### Microscopy, Infrared Spectroscopy, Population Study

## B92 An Evaluation of Five Methods to Develop Latent Prints on Thermal Paper

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**Learning Overview:** After attending this presentation, attendees will understand the additional difficulties thermal paper poses to latent fingerprint development, current development methods used, as well as benefits and drawbacks of these methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing further information concerning the development of latent fingerprints on thermal paper. Specifically, five development methods will be discussed: sequential application of 1,2-indanedione and ninhydrin, heat methods (oven, hot water immersion, and hairdryer), and p-Dimethylaminocinnamaldehyde (PDMAC) paper. These methods will be quantitatively and qualitatively compared to determine if a better method of development than those currently used exists.

Thermal paper, a type of paper that produces black or colored print in reaction to the application of heat, is commonly used for shop receipts, bus tickets, ATM receipts, and other applications. In the context of forensic science, the visualization of fingerprints on these receipts can connect possible suspects to a crime scene or time of evidence. Typical analysis of paper evidence involves ninhydrin and/or 1,2-indanedione dissolved in a polar solvent such as acetone or ethyl acetate, respectively; this poses a problem with thermal paper, however, because polar solvents dissolve the developer and allow it to react with the leuco dye to turn the paper black. These processes are often followed by the application of heat, which further activates the dye. If thermal paper turns black from these undesirable reactions, not only are the fingerprints less likely to be visible due to the loss of contrast, but the evidentiary print on the receipt may be lost entirely.

This experiment has two main components: analysis of fingerprints experimentally deposited on receipts and fingerprints naturally occurring on receipts. In the first part of the experiment, ten receipts from Kroger® and ten receipts from Costco® Wholesale were subjected to each development method. Results were photographed and rated (using a five-point quality scale) by three individual examiners. Statistical analyses used to determine the methods offering the most promising results were then applied to receipts with naturally occurring fingerprints. This ensured that the processes were effective at developing imperfect prints of varying ages. Preliminary results suggest PDMAC paper as an effective substitute for current methods since, unlike ninhydrin, no background darkening of the receipt paper is produced. The process itself is simple, although the receipts must remain sandwiched between the impregnated paper for 30 minutes to 24 hours; this time requirement, while lengthy, is comparable to the current 1,2-indanedione and ninhydrin time commitment. The resulting fingerprints are also comparable in quality to those visualized with 1,2-indanedione. In contrast, fingerprints developed from the other methods were not of high quality, if detected at all.

Future research should be conducted into PDMAC paper's specific mechanism to determine the length of time receipts should be sandwiched to produce the best visualization of the fingerprints. With this knowledge, the overall ease and effectiveness of developing latent prints on thermal paper will be improved, thus leading to more accurate comparisons.

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### Latent Prints, PDMAC, Thermal Paper



**B93 The Impact of the Ultraviolet (UV) Region and Plane Polarized Light (PPL) Microspectrophotometry (MSP) in the Characterization and Differentiation of Black Automotive Fibers**

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**WITHDRAWN**

## B94 Processing Gaps for 1,2-Indanedione (IND)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the effectiveness of IND and how it compares to other chemical development processes used on porous surfaces potentially bearing latent print evidence. This study also examined latent print development on the non-adhesive side of a variety of commonly used tapes and untreated wood samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a more in-depth understanding of the capabilities of IND while encouraging further research into using this in conjunction with other chemical detection methods. This research has determined that IND can further increase sensitivity of detection with the addition of metal salts such as zinc chloride. Adding zinc chloride helps produce better quality fingerprints on porous surfaces than Ninhydrin (NIN) and 1,8-Diazafuloren-9-One (DFO). The benefit of this research may result in an optimal processing sequence for certain unusual substrate surfaces.

Porous surfaces, such as paper, wood, and other cellulose-based materials, tend to absorb water and water-soluble residues found in sweat quickly after deposition. The majority of prints found on these types of surfaces are invisible to the naked eye, which requires specific methods to visualize them. Best practices have typically involved reagents such as NIN and DFO for the detection of latent prints on porous surfaces. However, these reagents do have limitations during latent print development. NIN has often been the method of choice, but it is not as sensitive and does not contain the fluorescent quality produced after development with DFO. Over the past decade, new chemical development methods and technologies have helped improve the quality and increase the specificity of latent print processing on porous surfaces. These were also investigated.

This two-phase study focused on creating an optimal processing sequence for IND. Phase 1 surveyed the success of latent print development on colored and textured substrates using IND, followed by an appropriate enhancement method. Phase 1 was not useful on untreated wood and the non-adhesive side of tapes. These substrates were then moved to Phase 2 for further testing. This focused on the evaluation of four detection sequences for these surfaces: (1) IND-Zn followed by NIN, (2) NIN followed by IND, (3) cyanoacrylate fuming followed by magnetic powder, IND, and Radar Absorbing Materials (RAM) (semi-porous sequence), and (4) only magnetic powder.

In Phase 1, a latent print examiner visually assessed the quality of print and ranked the best and worst processes for each photographed quadrant of the examined substrates after development. The quality of the prints was assessed using Metric 1, followed by ranking the best and worst process indicated by using Metric 2. In Phase 2, the best photograph for each development sequence was provided to the examiner for comparison, using the same metrics. The data was analyzed with a Pearson Chi-square statistical test and significant differences determined when p-value < .05.

NIN did not develop any detectable latent prints after IND for untreated wood, masking tape, and blue painter's tape. After IND was applied, the other chemical processes did not show any enhancement. IND worked the best on both wooden substrates compared to NIN. The semi-porous sequence, and the magnetic powder sequence worked the best on the non-adhesive side of the blue painter's tape. Magnetic powder worked the best on masking tape. RAM did not do well with any substrate. Overall, IND did the best on all substrates, except masking tape. Lastly, it was observed that the intensity of fluorescence on the masking tape was lost within seven days compared to the other processes, which did not lose any staining intensity over time. Further research will be done to determine retention quality of fluorescence after IND treatment and shelf life of the reagents.

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### Indanedione, Porous Surfaces, Latent Prints

## B95 The Development of Systematic Approaches for Physical Fit Comparisons of Trace Materials

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**Learning Overview:** After attending this presentation, attendees will be informed about newly developed systematic methods for evaluating performance metrics of physical fits of duct tapes and textiles and the inter- and intra-examiner variation in reported similarity scores.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the value of physical fits in trace evidence and the need for standardized, material-specific protocols that describe relevant distinctive features and consensus-based criteria. This presentation will demonstrate the development of methods for comparing edges of trace materials and highlight quantitative and statistical results that can offer additional support to inform an examiner's expert opinion.

When separated items of evidence can be realigned with distinctive features, the result is known as a physical fit. In trace evidence, physical fits represent the highest degree of association. Many laboratories consider the presence of a physical fit to be conclusive support regarding the common origin of the items. If a fit is identified and documented, oftentimes no further analysis is required. However, there are currently no consensus-based criteria or methods that can be used to support the reliability or probative value of a physical fit. This work expands on the research presented at recent American Academy of Forensic Science conferences on the development and validation of systematic protocols for comparing edges of various trace materials. This study assessed the performance of comparison methods for duct tapes and textiles to provide objective, reproducible, and quantitative similarity scores that can be used to evaluate the suitability of a fractured-edge comparison and describe the quality of a given fit.

The duct tape dataset consisted of over 2,500 duct tape edges of varying grade, separation method, and degree of stretching. Edge Similarity Scores (ESS) 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. Scores above 80% were found to support the conclusion of a match, while scores below 20% were indicative of a non-match. Intra-examiner and inter-examiner variation demonstrated agreement in reported ESS within 15%.

The textile dataset was composed of 100 comparison pairs, originating from five different clothing items with varying composition and construction. Twenty pairs were generated per item with ten each of stabbed and hand-torn fractures. Two examiners blindly compared all the pairs in each set and documented any relevant distinctive features that informed their opinion. In addition, each pair was examined through ten units taken as equal divisions of the total fracture edge length to document and calculate the ESS. The method's accuracy ranged from 93%–95%, with false negative rates between 4%–8% and the false positive rate lower than 2%. Intra-examiner variability following a second, blind comparison of the original set was generally less than 15%, and true matching pairs were more likely to receive similar or higher scores, while true non-matching pairs typically received similar or lower scores. More misclassifications were noted in the clothing items with fiber types of higher elasticity and in the hand-torn set, indicating that fabric type must be considered in the assessment of the suitability for physical fit comparisons, particularly if large levels of distortion and stretching are observed during the separation.

Overall, these comparisons demonstrated the importance of developing material-specific features and criteria for a physical fit evaluation. The systematic method allows for direct, blind comparison between examiners and provides a quantitative score that can be used to support the examiner's opinion and facilitate the peer-review process.

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### Physical Fit, Similarity Scores, Systematic Comparison





## B96 The Development of Odor Profiling Methods for the Detection of Contraband Firearms

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**Learning Overview:** After attending this presentation, attendees will better understand the Volatile Organic Compounds (VOCs) emitted from the actual firearm device in terms of the odor signature of firearm-related particulates for better source identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating volatile odor signatures from firearms to include the full device and other parts, such as magazines, because there is a need within the national security community to formulate an educated approach to understanding and enhancing canine detection of body-worn/carried firearms. This is a direct Organization of Scientific Area Committees (OSAC) need defined by the Dog and Sensors Subcommittee.

Firearms are recurrently the lethal means in forensic casework ranging from homicide and suicide to accidental injuries, indicating an important law enforcement and national security concern. With regard to mass shootings in this country, prevention tactics such as firearm detection canines have been at the front line of security tools for law enforcement personnel. For optimal use and field applications, canines need to be trained with high-quality training aids that are on par with current national security threats. Currently, there is no scientific foundation as to the chemical odor signature of firearms used in canine detection. Although previous studies have been focused toward an understanding of gunshot residue components as it relates to firearm discharge events, this study is focused on using the firearm as a direct source for odor profiling for purposes of body worn/concealed weapon detection. Creating an additional capability for firearm detection and analysis via odor profiles will allow unique odor signatures to be identified for instrumental classification.

This study provides a novel method application to generate an odor profile using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) as a rapid system for the analysis of headspace odor volatiles. Instrumental evaluation utilized Divinylbenzene/Carbon/Polydimethylsiloxane (DVB/CAR/PDMS) -coated SPME fibers that were injected into a GC/MS system for the identification of extracted volatile odor profiles from magazines (loaded and unloaded) as well as full firearm devices. Eight ammunition samples were collected and analyzed to provide another target analyte for analysis as a control. The magazines and firearms will be utilized from the local law enforcement shooting range. There were at least 30 unloaded and loaded magazine samples and 15 firearm samples. The findings include an assortment of compounds emitted from the magazines and/or firearms exhibiting distinctive odor profiles that have not yet been reported as part of organic gunshot residue constituents or similar discharge components in the literature. There is a dearth of research in terms of firearm odor volatiles, specifically as it relates to associated vapor signatures to enhance detector development in a variety of threats including terrorist attacks and mass shootings. This research responded to that gap by testing the firearm as a target sampling source to investigate the odor bouquets created before it is used in a shooting. The understanding of key odorants above a firearm will have a direct impact to national and international detection industries by providing knowledge that can be used to enhance training regimens, training-aid development to better prepare canine teams for current threats in our communities, and at ports of entry, as seen with the illegal transportation of firearms.

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### Volatile Organic Compounds, Canine Detection, Contraband Firearms

## **B97 What's That Lingering Smell? An Evaluation of Residual Decomposition Odor Volatiles in Colombian Territory**

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**Learning Overview:** After attending this presentation, attendees will better understand the Volatile Organic Compounds (VOCs) emitted after decomposed porcine remains have been removed from soil. Examining the quality and quantity of residual decomposition odor over time helps to address search and recovery methods of victim remains cases that Colombian law enforcement commonly encounter.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aiding in the knowledge and understanding of volatile odor patterns from residual decomposition odor sources at various decomposition times, support locating holding sites, and possible modes of transportation.

In 2019, Colombia averaged 32.8 homicides a day and many of these cases remain unsolved due to lack of evidence. By furthering the understanding of decomposition changes as measured by VOC profiles over time in soil matrices, enhanced methods can be developed to discover dump sites that can aid in a forensic investigation. To maximize the evidence recovered in crime scene response operational scenarios, human remains detection canines need to be trained to a variety of decomposition odor changes, including residual odor instances where the actual cadaver may have been removed from the search site. Currently, there is limited scientific research into how decomposition compounds change as a function of contact time between the decomposing substrate and the deposition environment. Emerging research has begun to look into the compounds present in soil during decomposition, but none are specifically looking at the quality and quantity of the volatile odor pattern over time, after the removal of the decomposing odor source, in a tropical, high altitude region such as Colombia.

This study provides novel data to generate a decomposition odor sequence using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) as a rapid system for the analysis of headspace odor volatiles. Instrumental evaluation utilized Divinylbenzene/Carbon/Polydimethylsiloxane (DVB/CAR/PDMS) -coated SPME fibers that were injected into a GC/MS system for the identification of extracted volatile odor profiles from the soil. The sample collection process consisted of allowing pigs to decompose for a period of 24 hours, 72 hours, 120 hours, and 30 days, then removing the pig and sampling the soil. The soil samples were sampled individually in 10mL glass vials, shipped on dry ice and allowed a period of 24 hours for headspace equilibration, then extracted via SPME methodology. Weather conditions such as temperature and humidity were recorded during each soil sample collection, as well as the pH and moisture content of the soil sample matrix. The findings include an assortment of VOCs emitted from each set interval, each interval exhibiting distinct odor profiles depending on the surface contact time of the decomposing odor source before removal of the pig. The benefit produced by the study has heightened the understanding of how residual decomposition odor changes over a set period of time in a tropical, high-altitude environment. This research will ultimately aid in the knowledge and understanding of volatile odor patterns from residual decomposition odor sources at various decomposition times, which will support in locating holding sites and possible modes of transportation.

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### **Volatile Organic Compounds, Residual Odor, Soil**

## B98 Optimization Parameters of Fragmentary Voltage and Collision Energy for the Identification and Separation of N-Methyl-N-(2,4,6-Trinitrophenyl)nitramide (TETRYL) 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 Kosovo Agency for Forensic Achievements 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 a 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, the explosives are divided into: high and low explosives based on the type and speed of the action they are doing. 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 TETRYL, 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 pictograms (pg) or even in femtograms (fg).

Therefore, considering the needs reasonable we have begun to create optimal parameters for Collision Energy (CE) and Fragmentary Voltage (FV) for identifying and dividing TETRYL from other explosives by using the 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.

Studies have found optimal CE and FV values for the identification and separation of TETRYL from other explosives, the change of fractional energy values greatly influences the fragmentation of the molecule being studied, and the fragmentation fractions molecule at all levels. This facilitates the identification of the molecule of unknown explosive and all fractions comply with the preliminary knowledge of the TETRYL structure and knowledge of its potential fragmentation, in addition to the excellent division achieved with this technique and the limit of detection up 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. In this study, the samples analyzed were organic explosives: 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, 1,3,5-Triamino-2,4,6-Trinitrobenzene (TATB), 1,2-Dinitroxyethane (EGDN), 1,3,5-Trinitrobenzene, 1,3-DNB (1,3-Dinitrobenzene (1,3,5-TNB), 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-Nitrotolueni (2-NT), 4-Nitrotolueni (4-NT), 2,2-Bis[(Nitrooxy)Methyl]Propane-1,3-Diyl Dinitrate (PETN), 3-Nitrotolueni (3-NT), 3,3-Dimethyl-1,2-Dioxacyclopropane (TATP), and Carbamite; the creation of methods for identification and separation of explosives by doing the optimization of parameters on LC and MS/MS.

This study attempted to create methods just for explosives like HMX, RDX, TATB, TETRYL, TNT, 2,6-DNT, HNS, PETN and carbamite. The two methods created are: EKSPLOSIVES-MMI-APCI.m and EKSPLOSIVES\_2-6-2-4\_DNT\_MMI-APCI.m.

Parameter optimization for both methods are as follows: flow of mobile phase 0.5mL/minute, temperature of colon 35°C, length of colon 3x50mm, diameter 3.5µm, ionization source Multimode (MMI), ionization model (APCI), volume of sample injected 20µm. Mobile phase was suitable for both above methods.

For the above explosives, this study has conducted optimization of parameters as FV and CE for the identification and separation of explosives in a trace amount until fg.

### TETRYL, Collision Energy, Fragmentary Voltage

## **B99 Characterization of Ignitable Liquid Residues and Interfering Pyrolysis Products of Common Substrates in Forensic Fire Debris Analysis**

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**Learning Overview:** The goals of this presentation are to help attendees regarding: (1) the correct identifications of the accelerants and their residues from commonly encountered fire debris samples; (2) the retainability of the ignitable liquids by different substrates at the fire scenes; (3) the Pyrolysis Products (PyPs), if any, originating from the substrates that may be wrongly interpreted as the residues of ignitable liquids in the fire/ arson cases; and (4) the retaining ability of the traces of Ignitable Liquid Residues (IGLRs) from different accelerants (Gasoline, Kerosene, and Diesel [GKD]) at the fire/ arson scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight to correctly interpret the IGLR case samples in the presence of substrate interference PyPs.

Forensic investigation of fire and arson cases is an emerging field in Pakistan. A characterization of the traces of the ignitable liquids (GKD) and their IGLRs from the debris collected from the fire/ arson scenes is needed. The incorporation of the false positive identifications of the IGLRs from the debris due to the PyPs of the substrates was studied. The substrates commonly available in the houses, offices, and community areas of Pakistan that had never been examined for forensic purpose were selected for this study.

Neat ignitable liquids (GKD) were diluted with n-pentane to prepare a solution of 2.5 $\mu$ L/mL. A mixture of equal quantities of GKD was diluted with n-pentane to obtain a concentration of 2.5 $\mu$ L/mL. The concentrated n-pentane extracts of neat 19 substrates, unburnt substrates, and burnt substrates (with and without IGL) were prepared for analysis. The analysis was performed using gas-chromatograph (7890A) mass spectrometer (5975C) with electron impact ionization applying full scan mode.

The pre-burning examination of substrates showed no interfering peaks that may be confused with IGLR, whereas some burnt substrates showed the limited amount of PyPs similar to IGLRs (n-alkanes, cycloalkanes and alkyl aromatics). The type and surface texture of 19 substrates also imparted their role regarding the retainability of IGLRs in their post-burning debris. This study also clarified that the chemistry of ignitable liquids also played an important role in leaving their traces at the fire/ arson scene.

This study concluded that the intact substrates did not show any interfering peaks, whereas the post-burning examination of the substrates revealed the absence of interfering PyP similar to IGLR in most substrates. Very few substrates interfered but produced a limited number of compounds that could be ruled out using their controls in analysis as well as by following the American Society for Testing and Materials (ASTM) 1618–19. The overall identification of IGLR was not affected by the PyPs of substrates. The IGLR persistency comparison of GKD from post-burning debris showed that kerosene and diesel (medium to heavy petroleum distillates) left more traces as compared to the gasoline.

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### **Ignitable Liquid Residues, Substrates, Pyrolysis Products**

## **B100 Shotgun Hunting Fatalities—How to Be Wrong in Reconstruction! Pitfalls for the Medical Examiner and the Ballistics Expert: A Case Report**

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**Learning Overview:** After attending this presentation, attendees will be aware of the importance of the collaboration of a medical examiner and a ballistic expert in the reconstruction of the dynamics of a hunting accident.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usefulness of combining forensic ballistic investigations and the remaining investigations, such as on-spot surveys, testimonial collections, and examination of bullets and cartridges, in order to provide a motivated, scientifically valid and therefore concretely usable hypothesis in legal framework.

In gunshot casualties, the only analysis of biological data may be insufficient for reconstructive purposes. In fact, the reconstruction of the events that had made use of a firearm is a problem that apparently escapes the exclusive pathological study of ballistic agent injuries and requires an integrated assessment by others criminalistic and ballistic surveys.

The purpose of this presentation is to present the case of a 60-year-old male hunter dead in a hunting accident that took place in November 2018 in Tuscany, Italy. During a boar hunt inside an olive grove, a position line was set up, on whose trajectory, at a distance of about 40 meters from each other, five hunters were positioned. The current legislation on hunting wild boars stated that hunters could fire only in front of them. In the event that the boar had passed the same line, it was mandatory to secure the gun and rotate 180° before aiming the gun again, thus avoiding hitting the hunters placed on the same line. At the sight of the boar, some of the hunters fired a total of four rifle shots. Once the sequence of shots was over, one of the hunters, C.F., was found dead on the ground. One of the hunters, F.L., aware of having fired without respecting the rules, pleaded guilty. The results of the medical legal investigation (site inspection, body examination) were influenced by the self-accusation of F.L.; in fact, they lacked the fundamental elements such as surveys, the analysis of weapon bullets and used cartridges, orography, and bullet penetration and F.L. was sentenced.

In a second-degree criminal trial, the analyses, carried out later, instead evaluated all the elements left out by the technical consultant; in particular, the shot and bullet distance were studied. They were incompatible with the respective positions of the firing, presumed shooter, thus excluding the responsibility of F.L.

It should be remembered that in assessing the firing distance, the evaluation of the penetration of those bullets in the various biological structures crossed by the bullets is of primary importance. In this case, the distance between the victim and the shooter was studied using Sellier's formulas. It was calculated that the bullet exploded at a distance of 40m; that is the distance between C.F. (the victim) and F.L. (alleged shooter) that should have had a penetration capacity so that it went out of the body. On the contrary, the external body examination showed the presence of the bullet held in correspondence of the scalp in the temporal region. Therefore, the presence through a blind bottom, with the retained bullet, indicates that the bullet could not have exploded at a distance of less than about 100m.

Another important element taken into consideration was the one of the height difference between the two hunters, which was six meters, obtained by taking into consideration the orographic elements.

Therefore, based on these results, together with the data provided by the autopsy, and with the integration of the ballistic survey investigations, it has been shown that the distance between the victim and the shooter had to be greater than 100m and that the difference between the mutual positions of firing and shooting excluded that the shot had been fired by the F.L. hunter.<sup>1-6</sup>

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### **Ballistics Expert, Shotgun Injuries, Formula Sellier**

## B101 An Adhesive Analysis of Various Tapes Using Laser-Induced Breakdown Spectroscopy (LIBS)

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**Learning Overview:** After attending this presentation, attendees will better understand LIBS and how it can be applied to adhesive analysis to aid in forensic investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing how LIBS can be a useful tool for adhesive analyses.

Adhesive tapes are commonly found in crime scenes. Any tape has adhesive on one or both sides. This adhesive can be left as residue when the tape comes into contact with other surfaces. Although it is not always considered the highest priority, this can often be interesting evidence in forensic cases as it still often has value in completing a full profile of the crime. The adhesive can be instrumental in differentiating between the different varieties of tape. This can include both different types of tape, such as duct tape or electrical tape, but also between different brands of the same type of tape. The most common type of tape used in crimes is duct tape, with the second being electrical tape. Other minor types of tapes found in crime scenes include packaging tape, filament tape, and masking tape. Once tape or tape residue is found at a crime scene, it is the forensic scientist's job to determine the type of tape and to further determine with certainty that it is the same tape that was found at other places (i.e., usually, with the person under investigation).<sup>1</sup>

LIBS is a spectroscopic technique in which a high-powered laser is focused onto a surface. The focused laser pulse heats, ablates, atomizes, and ionizes the surface material and results in the formation of a plasma. The plasma light is collected, spectrally resolved, and detected. Elemental emission is observed in the form of a spectrum. The LIBS spectrum can provide both qualitative and quantitative information. LIBS has many applications including space, environmental, explosives, forensics, etc.<sup>2-5</sup>

For this study, LIBS was used to analyze the adhesive side of various tapes to determine its feasibility for unique adhesive detection. The tapes analyzed included various brands of duct tape, electrical tape, masking tape, packaging tapes, filament tapes, and medical tapes. LIBS data was taken on the adhesive side of all of the tape analyses. Spectra were collected from 220 to 950nm. The spectral data was analyzed to determine unique spectral lines for the various tapes analyzed. The data shows that there are differences in the adhesives of the various brands of duct tape. Furthermore, duct tape and electrical tape were able to be distinguished from each other. Useful spectra were not obtained on many of the clear tapes analyzed due to coupling issues with the focused pulse and the clear surface of the tape face. However, spectra were obtained for many tapes with an opaque or colored tape face.

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### Adhesive Analysis, LIBS, Atomic Spectroscopy

## B102 The Application of Laser-Based Methods for the Analysis of Gunshot Residue (GSR) Originating From Modern Ammunition

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**Learning Overview:** After attending this presentation, attendees will understand the creation and characterization of inorganic GSR standards as well as the capabilities of Laser-Induced Breakdown Spectroscopy (LIBS) and Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) to offer rapid and reliable screening methods for shooter and non-shooter hand samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with two laser-based techniques that can be used for GSR hand analysis and expanded to other sample types such as facial areas, hair, vehicle interiors, clothing, and glass to enhance laboratory workflow and informative value.

GSR is a common form of trace evidence submitted to crime laboratories in firearm-related crimes that pose a risk to public safety. Even though investigation of firearm-related crimes requires fast leads, a GSR report from a crime laboratory can take weeks or months to produce, which can impede fast decision making and delay arrest of suspected individuals. Therefore, the field may benefit from the development of new methods for rapid screening of GSR evidence to provide law enforcement and the judicial system with complementary tools to improve case management.

A research group has developed laser-based methods, including LIBS and LA-ICP/MS, to address this need for rapid screening of GSR samples. The two promising methods were applied to tailor-made Inorganic GSR (IGSR) standards and to residues collected from hand samples from both non-shooters (background population) and known shooters using modern ammunition (leaded and lead-free) and different firearms. The central hypothesis of this research is that the use of LIBS and LA-ICP/MS will provide complimentary analytical techniques for detecting elemental compositions of IGSR standards, as well as rapid screening and classification of shooter samples based on the presence or absence of IGSR elemental profiles from modern ammunitions.

IGSR suspensions created using eight different primer types, four leaded (Remington®, Sellier & Bellot®, TulAmmo®, and Winchester®) and four non-leaded (CCI®, Fiochi®, Inceptor®, and Syntech®), were analyzed by both instrumentations to establish baseline compositions. Also, 64 known-shooter samples (37 leaded and 27 non-leaded), and 60 non-shooter samples were analyzed by both LIBS and LA-ICP/MS to test the methods' abilities to correctly identify IGSR from shooter samples and evaluate the thresholds of the elemental profiles on background samples. The LIBS method completed the screening in one-and-one-half minutes per stub, while the LA-ICP/MS method was nine minutes. Therefore, LIBS was used to process all stubs collected from hands, while LA-ICP/MS was performed on shooter and non-shooter stubs previously positive by LIBS. Both methods incorporated spatial information by collecting 25 individual spectrum from a region of 100µm in diameter. For leaded ammunition, emission lines for Pb (405.8nm, 368.3nm) Ba (455.4nm, 493.4nm), and Sb (252.8nm, 259.8nm) were monitored during LIBS analysis while isotopes of lead (<sup>208</sup>Pb, <sup>206</sup>Pb), barium (<sup>138</sup>Ba, <sup>137</sup>Ba), and antimony (<sup>121</sup>Pb, <sup>123</sup>Pb) were monitored for LA-ICP/MS. A more extensive elemental list was included for non-leaded ammunitions: Bi (293.8nm, 472.2nm), Cu (324.7nm, 327.4nm), Ti (334.9nm, 376.1nm), Zn (334.5nm, 328.2nm), K (766.4nm, 769.9nm) for LIBS, and bismuth (<sup>209</sup>Bi), copper (<sup>65</sup>Cu, <sup>63</sup>Cu), titanium (<sup>48</sup>Ti, <sup>49</sup>Ti), zinc (<sup>64</sup>Zn, <sup>66</sup>Zn), and potassium (<sup>39</sup>K, <sup>41</sup>K) for LA-ICP/MS.

The methods identified the elemental composition in all IGSR suspension samples, while LA-ICP/MS provided higher sensitivity and selectivity and consistently had more positive spots in each pattern. The resulting baseline compositions were: Remington® (Pb, Ba, Sb), TulAmmo® (Pb, Sb), Sellier & Bellot® (Pb, Ba, Sb), Winchester® (Pb, Ba, Sb), Fiochi® (Bi, Cu, K), Syntech® (Bi, Cu), Inceptor® (Cu, Ti, Zn), and CCI® (Ba). A critical threshold of the mean plus three times the standard deviation of S/N ratios in the background population was used for determining the presence or absence of elements in shooter samples. Shooter samples were broken down into three different categories of standard shooters (leaded elements), non-leaded shooters (non-leaded elements), and mixed shooters (leaded and non-leaded elements). LA-ICP/MS resulted in true positive rates of 91% for standard shooters and 78% for non-leaded and mixed shooters, while LIBS resulted in true positive rates of 78% for standard shooters and 45% for both non-leaded and mixed shooters.

Laser-based methods applied in this study demonstrate the feasibility of new techniques for the screening of GSR. LA-ICP/MS had greater sensitivity, but LIBS has the advantage of being field applicable due to its portability. Both methods improved analysis time of IGSR and could be expanded to other sample types such as facial areas, hair, vehicle interiors, clothing, and glass to enhance laboratory workflow and informative value. Continued development of rapid techniques could assist in providing fast decision making and probabilistic assessment of the evidence needed during the investigation of firearm-related crimes and interpretation of evidence in court.

### Laser Ablation, Gunshot Residues, Lead-Free Ammunition

## **B103 The Application of Wavelet Transform and Transfer Learning for Gasoline Classification Using a Hand-Held Raman Spectrometer**

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**Learning Overview:** The goal of this presentation is to provide attendees with a practical solution for discriminating between gasoline and non-gasoline samples using a hand-held Raman spectrometer. The application of wavelet transform for Raman scattering signals will be discussed in this presentation. This study will also demonstrate how transfer learning can be applied to develop Artificial Intelligence (AI) to detect gasoline samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new approach for effective detection of gasoline by adopting wavelet analysis and deep learning for Raman spectra processing. A machine learning model for distinguishing gasoline from seven common Ignitable Liquids (ILs) was developed based on deep learning algorithms. The model developed in this work could predict accurately for gasoline and non-gasoline liquids using a hand-held Raman spectrometer for signal collection.

Gasoline is one of the ILs that has been most widely used as an accelerant to speed up the escalation of fire in arson cases. A rapid and accurate approach to detect gasoline allows investigators to recognize and preserve the evidence in time correctly and get a head start tracing the prime suspect during the investigation. A hand-held Raman spectrometer combined with AI is an attractive analytical platform that offers a rapid and field-deployable capability for precise gasoline and non-gasoline ILs classification.

In this work, gasoline with three grades (regular, mid-grade, and premium) and seven different ILs (charcoal lighter, pure gum spirits of turpentine, kerosene, mineral spirit, hexane, xylene, and toluene) were chosen for AI development. A hand-held Raman spectrometer with a red 785nm laser wavelength was utilized to acquire 130 gasoline Raman spectra and 105 non-gasoline liquids Raman spectra. One mL sample was placed in a glass vial for Raman signal measurement. All Raman spectra of the samples were acquired under various conditions such as measuring under ambient light and without ambient light. Each spectrum was obtained with 20 seconds of integration time and has been baseline-subtracted to correct the background signal. The spectral range was recorded from 400 to 2,300 $\text{cm}^{-1}$  for analysis. The features of Raman spectra were processed by the Continuous Wavelet Transform (CWT) to create the scalograms, representing the absolute values of the CWT coefficients of the signals in the spectra. Followed by the data processing, a convolutional neural network for transfer learning was adopted to create a classifier to distinguish between gasoline and non-gasoline ILs based on the pre-trained neural network architecture. In the architecture, 144 layers acted as filters to identify common and specific features of the scalograms to be re-trained for recognizing scalograms from gasoline and non-gasoline. During transfer learning, 235 scalograms were randomly divided into two groups: 80% of the images (188 scalograms) for training and 20% of the images (47 scalograms) for model validation.

From the training progress, it was found that the constructed model successfully reached 100% accuracy in classifying gasoline and non-gasoline ILs within 2 epochs in a 20-epoch training cycle. The validation accuracy was reported as 100% accurate as well. This study demonstrated a new approach for gasoline classification based on Raman spectra using wavelet analysis and deep learning. No normalization of the spectra was required for AI development. Through this new approach, a trained AI model capable of rapidly and accurately detecting gasoline with different grades from seven other ILs was developed and successfully validated.

### **Hand-Held Raman Spectrometer, Wavelet Analysis, Deep Learning**



## B104 Revisiting the Recovery of Defense Sprays From Fabrics

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**Learning Overview:** After attending this presentation, attendees will be able to describe a new extraction method for the recovery of defense sprays from clothing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing attendees how they could easily implement and verify a method for the analysis of trace amounts of defense sprays in casework.

Recent mass gathering events have resurged an interest on the forensic analysis of defense sprays. On the one hand is the interest of individuals to sustain the claim of exposure; on the other, the interest from law enforcement to sustain the claim that a defense spray was used before escalating force. Previously published work on the extraction of defense spray from clothing used a variety of solvents for liquid solvent extraction such as hexanes, methanol, ethyl acetate, diethyl ether, chloroform, and dichloromethane, as well as Solid-Phase Micro Extraction (SPME).<sup>1-5</sup> For the analysis, Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) have been the techniques of choice.<sup>1-5</sup> When using direct liquid solvent extraction, the recoveries of capsaicin and dihydrocapsaicin reported were less than 60% on unwashed cotton samples and less if the fabrics were manipulated (e.g., washed).

For this study, a method using liquid extraction with toluene was developed to recover residues of capsaicin and dihydrocapsaicin from fabrics followed with the analysis by GC/MS. For all experiments, pieces of 3x5cm of different fabric types such as cotton, polyester, and nylon were used. For the recovery study, the fabric pieces were spiked with standards of capsaicin and dihydrocapsaicin at different concentrations and were allowed to dry in the laboratory. All fabric pieces were extracted with toluene using a rotating mechanical shaker for at least two periods of one hour. An aliquot of the extracts was transferred to GC vials and *n*-tetracosane-*d*<sub>50</sub> was added as internal standard. The samples were then derivatized with *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) for about 24h at a mild temperature of 45°C in an aluminum block heater. When it was not possible to begin the analysis within that 24h window, the samples were stored in a deep freezer (-20°C). The method was also implemented for the analysis of ten defense sprays, neat and on different fabrics of cotton, polyester, and nylon. The results of the analysis of the neat sprays were compared with each other and with the results from spiked fabrics. The study was completed with a small persistence study of the target compounds on the spiked fabrics for a period of two months.

The prevalence of the non-volatile fraction in the neat defense spray content made discrimination using their composition profile a challenge. Nevertheless, the method rendered high recoveries of capsaicin and dihydrocapsaicin of >90% with small limit of quantitation in the order of 4ng. These results were observed across fabrics and within two weeks before extraction, with some decrease thereafter.

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### Defense Sprays, Pepper Spray, Capsaicin

## **B105 The Detection of Lead-Free Inorganic and Organic Gunshot Residue (IGSR and OGSR) Using Laser-Induced Breakdown Spectroscopy (LIBS), Electrochemistry, and Machine Learning**

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**Learning Overview:** After attending this presentation, attendees will be able to identify and understand the differences between standard and non-toxic ammunition and the analytical challenges in analyzing GSR.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the characterization and detection of non-leaded GSRs by spectrochemical and electrochemical methods for screening purposes. The combination of both methods improves the accuracy and reliability of IGSR and OGSR analysis.

Current changes in ammunition formulations require the inclusion of emerging GSR methods that could deal with newly manufactured lead-free residues. The current gold standard for GSR analysis is Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDS), which identifies IGSR elements like lead, barium, and antimony.<sup>1</sup> However, some compositions contain only low atomic number elements that may become more challenging for the automated discovery and identification of GSR particles by SEM/EDS. Therefore, the detection and classification of these elements along with OGSR compounds can complement current practice. Techniques such as LIBS and Electrochemistry (EC) allow for faster identification and simultaneous detection of IGSR and OGSR. In this study, a micro-spot LIBS method was used to collect 25 spectra per stub with simultaneous multielement detection. An electrochemical sensor based on Screen-Printed Carbon Electrodes (SPCE) and square-wave anodic stripping voltammetry allowed for the detection of both IGSR and OGSR data in just a few minutes.

SEM/EDS, LIBS, and EC were used to characterize several non-toxic ammunitions. This presentation evaluates figures of merit such as Limit Of Detection (LOD) and peak resolution for both LIBS and EC methods. Authentic shooter samples were collected using carbon adhesive stubs from the front and back of shooters' hands. A previously collected background population was used for critical threshold comparison with the shooter population. This population set consisted of 100 non-toxic shooter samples and 150 background hand samples. The occurrence of selected elements and organic compounds are reported for the background population as a means to understand prevalence of these residues on the hands of individuals who have not been in the vicinity of a firing event.

In addition to critical threshold values, machine learning algorithms, including logistic regression and neural networks, were used to analyze the performance measures of true positive, true negative, false positive, and false negative rates in comparison to standard ammunition data collected previously by this group. Lower performance than standard ammunitions was seen in the non-toxic population by both LIBS and EC, with an accuracy of 89% and 79%, respectively. Moreover, the orthogonal detection method combining LIBS and EC data resulted in an improved accuracy of 98% on this dataset.

This research study successfully classified the known-shooter samples originating from leaded and lead-free ammunition, providing complementary tools for current methods.

### **Reference(s):**

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### **Gunshot Residues (GSR), LIBS, Electrochemistry**

## **B106 A Comparison of Sampling Techniques of Surface and Bulk Dental Enamel for Analysis With Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)**

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**Learning Overview:** The goal of this presentation is to address the efficacy of using laser ablation to sample dental enamel compared to previous methods used for surface and bulk enamel for the purpose of geolocation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining different procedures for sampling surface and bulk dental enamel for geolocation, which in turn can aid in the identification of unidentified remains in the state of Virginia.

There is a need in the forensic community for novel pathways through which to identify unidentified remains when other methods have failed. If the region where the individual lived could be identified, this could aid in potentially connecting the remains to a missing person's report from that region. In a previous study, it was shown that the trace isotopes in surface enamel are distinctly different from the bulk enamel in human teeth. This allows for more specific information about where the individual most recently lived, due to the demineralization and remineralization cycles of the outer enamel, and where they lived in their formative years from the bulk enamel that is formed first and not changed over time. Ultimately, the trace isotopic signatures can be collected from both surface and bulk enamel to develop a database of known signatures from known regions in Virginia, aiding in the identification of approximately 165 sets of unidentified remains currently in the Offices of the Chief Medical Examiner. The purpose of this project is to compare the previously established method of sampling both surface and bulk enamel for isotope analysis using ICP/MS and using laser ablation coupled with ICP/MS for more precise targeting.

To compare sampling techniques, each sample was first chemically etched using a nitric acid etching solution following procedures from the previous study and the resulting solution was diluted and analyzed using ICP/MS for  $^{24,25,26}\text{Mg}$ ,  $^{50,52,53}\text{Cr}$ ,  $^{56}\text{Fe}$ ,  $^{58,60,61,62}\text{Ni}$ ,  $^{64,66,67,68,70}\text{Zn}$ ,  $^{74,76,77,78,80,82}\text{Se}$ ,  $^{84,86,87,88}\text{Sr}$ ,  $^{130,132,134,135,136,137,138}\text{Ba}$ , and  $^{204,206,207,208}\text{Pb}$ . Each sample was then cut using a double-bladed diamond saw to remove a small section of the tooth from an area that was not chemically etched. The section was then sampled using laser ablation at the surface of the tooth, 100 $\mu\text{m}$  from the surface and 200 $\mu\text{m}$  from the surface three times for each sample. The samples were then analyzed using ICP/MS for the same isotopes as the etched surface samples. Finally, the bulk enamel was extracted from each sample by first breaking it with a hammer and then manually removing pieces of enamel that were then crushed into a powder using a mortar and pestle. A small quantity of the powder was then chemically digested and the resulting solution was diluted and analyzed for the same isotopes of the etched surface sample.

Preliminary discriminant function analysis indicates that samples can be classified by region, which is consistent with findings from the previous study. Additionally, preliminary comparisons of samples collected at 0, 100, and 200 $\mu\text{m}$  indicate that trace isotopes at 100 and 200 $\mu\text{m}$  from the surface are very similar, whereas the trace isotopes at 0 $\mu\text{m}$  appear to be discernable from those at 100 and 200 $\mu\text{m}$ . This allows for data collected at 100 or 200 $\mu\text{m}$  to be treated as equivalent to bulk enamel for direct comparison between the two collection methods. Further statistical analyses will compare the data from the samples obtained using chemical etching and manual extraction to the samples obtained using laser ablation, allowing for comparison of the efficacy of sampling techniques.

### **Geolocation, Isotope Analysis, Human Identification**

## **B107 The Digital Preservation of the President John F. Kennedy Assassination Bullet Artifacts**

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**Learning Overview:** After attending this presentation, attendees will have learned about bullet artifacts that were part of the John F. Kennedy assassination. Attendees will learn how the National Institute of Standards and Technology (NIST) digitally scanned these artifacts and made them available for the public to view and download. Some of the measurement challenges faced will be discussed, providing a better understanding of measurement techniques for fragmented bullets.

**Impact on the Forensic Science Community:** This presentation will impact the forensics science community by expanding knowledge of the John F. Kennedy assassination and describing 3D surface maps of the bullet artifacts that are available to download and view.

The assassination of President John F. Kennedy was a defining moment in United States history and has left a lasting impact on generations. As the nation's record keeper, the National Archives and Records Administration (NARA) is responsible for preserving many of the artifacts and documents that were part of the assassination investigation, including the recovered bullets. NIST has a long history of developing and producing the technology required to preserve important and priceless documents such as the Declaration of Independence, the Constitution, and the Bill of Rights. This history combined with expertise in areal surface topography measurements enabled NIST to assist NARA in digitally preserving the assassination bullet artifacts, and in doing so, facilitated public access.

In collaboration with NARA, NIST acquired high-resolution photographs and 3D models of the bullet artifacts. State-of-the-art confocal and focus-variation microscopes were used to scan each artifact and obtain 3D surface topography and color maps of the artifact surfaces. The lateral point spacing of the obtained maps ranged from 1.5 $\mu$ m to 4 $\mu$ m. The measurements were then combined into 3D models of the entire bullet surfaces. NARA has made these data sets available online to the public for download and interactive virtual inspection.

Several technical challenges in scanning the bullet artifacts will be discussed. The bullet fragments had extremely complex features, including reentrant surfaces. This required scanning the surfaces at many different artifact orientations and then assembling all the scans using advanced software algorithms. These algorithms registered the individual scans to each other in 3D space using overlapping measurement regions and then merged the scans together into a single 3D surface model of each artifact. The large number of high-resolution scans resulted in large amounts of data that had to be managed, both during the merging process and for computer visualization. In addition, there were challenges related to surface illumination during measurement to acquire the color information of the artifacts accurately. This was achieved by using a combination of illumination techniques, including coaxial and ring lighting.

This presentation will be divided into the following sections: (1) review of the project scope; (2) overview of the instrumentation, methods, and challenges in measuring the surfaces of the artifacts; (3) a detailed look at the image stitching and merging process to create the 3D models; and (4) demonstration of the final color 3D artifact models that have been made available to the public for download and virtual inspection.

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### **Kennedy, Assassination, Bullet**

## B108 A Validation Study of the Accuracy, Repeatability, and Reproducibility of Firearm Comparisons

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**Learning Overview:** After attending this presentation, attendees will better understand the design process and results achieved in a large-scale decision analysis (“black box”) study involving a large group of firearms examiners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing empirical measurements of the accuracy, repeatability, and reproducibility of analyses performed by firearms examiners for cartridge case and bullet sample sets.

The study hypothesis was that trained/qualified firearms examiners can accurately determine source conclusions (repeatability) when applying the Association of Firearm and Tool Mark Examiners (AFTE) Theory of Identification, as well as reproduce the same result(s) when later encountering the same comparison.

This study complements those conducted previously, adding important additional features. A previous study conducted on the accuracy of firearms examiners was generally viewed favorably by the President’s Council of Advisors on Science and Technology (PCAST), but PCAST advised that additional, similarly designed “black box” investigations were required to establish foundational validity.<sup>1,2</sup>

The present study implemented a fully randomized, open set, and double-blind design involving challenging comparisons of fired bullets and cartridge cases. In order to maintain double-blind conditions, the experimental study was conducted under contract by scientific staff at another organization, which sent randomized specimens to participating examiners and performed statistical analysis. Following a call for participants, volunteer active examiners were provided with 15 comparison sets of two known and one unknown cartridge cases fired from a collection of Beretta® and Jimenez® firearms and 15 comparisons sets of two known and one unknown bullets fired from Beretta® and Ruger® firearms. In order to minimize reproduction of marks, the ammunition selected for testing was Wolf Polyformance® 9mm Luger (9x19mm), with acrylic polymer-coated, steel cartridge cases and lead core, copper-plated, steel-jacketed bullets. The firearms and ammunition selected for this study were purposely chosen due to their propensity to produce challenging and ambiguous test samples creating difficult comparisons for examiners. The firearms, bullets, and cartridge cases used for the study were collected by researchers in the first laboratory and delivered to scientists in the contract laboratory, who then conducted the study and engaged in the generation and distribution of test packets and collection and analysis of the data. A total of 173 qualified examiners took part in the study. The participating examiners were asked to follow the provided instructions rather than adhere to their laboratory policies and were instructed not to discuss their results with anyone else in their laboratory. In order to further maintain the double-blind, “black box” nature of the study, the team associated with communicating with the examiners was not aware of the contents of each comparison set, and the experimental / analysis group was never aware of the examiner’s identities.

The total number of comparisons carried out was 20,130, of which 8,640 tested for accuracy, 5,700 tested for repeatability, and 5,790 were tested for reproducibility. Definitive false positive error rate estimates that take examiner heterogeneity into account are 0.66% for bullets and 0.93% for cartridge cases. False negative error rate estimates are 2.87% (bullets) and 1.87% (cartridge cases). These estimates are based on data that include comparisons from barrels produced sequentially in time and those separated in the manufacturing process, rounds fired early in the life of a barrel and after many rounds had been fired, and rounds fired from both high and low cost-point firearms. Individual error rates within each of these categories have also been calculated and vary slightly from the overall average in ways that might be expected (e.g., higher error rates are seen for rounds widely separated in firing order than sequentially, lower cost point firearms have a higher false negative error rate than average, etc.). As in earlier studies, the majority of errors were produced by a relatively small number of examiners. The numbers found in the current study are generally consistent with the results reported in prior studies and, therefore, constitute the foundational validation the PCAST report said was lacking.

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2. PCAST. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. ([https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast\\_forensic\\_science\\_report\\_final.pdf](https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_final.pdf): President’s Council of Advisors on Science and Technology, 2016).

### Firearms Examination, Error Rate, Black Box

## **B109 The Impact of Bullet Caliber and Composition on the Statistical Distribution of Non-Match Scores Based on High-Resolution Topography Measurements**

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**Learning Overview:** The goal of this presentation is to present the recently developed firearm identification method, based on high-resolution topographic data, for the comparison of fired bullets. Attendees will better understand the 3D profile-based (or pattern-matching) approach and the line-counting approach, as well as the systematic effects of different calibers and bullet compositions on the behavior of the statistical distribution of bullet similarity measures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding of the complementarity of the pattern-matching and line-counting perspectives for the analysis of bullet topographies. Additionally, this study will present the results that constitute an in-depth analysis of the systematic effects of bullet caliber and composition on the behavior of similarity measures.

High-resolution 3D acquisition technologies have been a catalyst for the development of quantitative analysis of toolmarks present on cartridge cases and bullets, with the aim of overcoming the limitations inherent in direct observation under a comparison microscope and reflectance (2D) image analysis. As most bullets have several regions of interest (Land Engraved Areas [LEAs]), a difficulty specific to the development of bullet comparison algorithms is the need to compare all available pairs of LEAs and then produce a score that quantifies the overall similarity of the two bullets being compared. It is therefore necessary to determine relevant similarity measures for LEA pairs and combine the resulting set of scores into a global similarity measure.

Over the past few years, a bullet comparison algorithm that considers the pattern-matching and line-counting approaches has been in development. Each LEA pair comparison produces four scores, two of which are profile-based (pattern matching), while the other two are based on line counting. The set of score values resulting from all LEA-to-LEA comparisons is used to determine the best phase between the two compared bullets. The Pattern Matching Score (PMS) is then defined as a combination of the best Cross-Correlation Function (CCF) and Absolute Normalized Difference (AND) values at this phase; similarly, the Line Counting Score (LCS) is defined as a combination of the best scores for peaks and valleys at this phase. The original analysis performed in 2018 using a dataset of 9mm copper-jacketed bullets enabled the definition of a False Match Rate (FMR) function on a 2D plot that shows both similarity scores for matches and non-matches.

To refine this study, 65 known match pairs were acquired using a 3D sensor based on a non-linear photometric stereo technique, for each of five calibers with conventional rifling: 9mm, .25 Auto, and .45 (copper-jacketed) and .22 and 357 (lead). All known match pairs were analyzed using a virtual comparison microscope prior to numerical analysis in order to define the subset of pairs that could actually be identified. The match and non-match statistical distributions of scores and the FMR function were for each dataset. This study found that the five non-match probability densities could be extrapolated with an exponential distribution where non-match data is missing and match data is available. A typical FMR value that discriminates between low and high levels of visual similarity was found to be 1/10,000 for each of the five calibers. Most visually matching pairs are found at FMR values lower than 1/10,000. A significant finding is the sensitivity of the non-match score distribution (and its corresponding FMR function) to the caliber and composition of the bullets. Research avenues are proposed to significantly reduce this variability, with promising preliminary results.

### **Firearm Identification, Bullet Topography Measurements, False Match Rate**

## B110 Estimating Muzzle-to-Target Distance From the Physical Characteristics of a Bullet Hole

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**Learning Overview:** After attending this presentation, attendees will have learned of a new method that relates bullet hole depth and damage characteristics to the muzzle-to-target distance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new method of analyzing muzzle-to-target distance that can overcome the limitations of traditional distance analysis tests.

Determining the muzzle-to-target distance of a firearm discharge is an integral part in crime scene reconstruction. When combined with the bullet's trajectory and impact angle, the shooter's location can be approximated, which can support or refute eyewitness accounts and suspect statements. The method most often employed during case work is the Modified Griess test, which analyzes Gunshot Residue (GSR) patterns around bullet holes. This test, however, has a weakness of a three- to five-foot range limit. As a chemical test, the evidence at the crime scene can also be contaminated or obscured. Physical visualization of GSR patterns may require instruments that may not be available at the crime scene. At the same time, bullet holes may be on surfaces that cannot be transported to the lab, such as on walls or doors. This study identified the problem that the Modified Griess test may not always be optimal at a crime scene.

This study sought to develop a new method that overcomes this range limitation as well as offer other unique advantages that the Modified Griess test may not provide. The goal was to observe the physical damage patterns of a bullet hole and relate that to the muzzle-to-target distance. It decided to test this on plywood and Medium Density Fiberboard (MDF), which are common wood substrates found as indoor building materials. Test fires were conducted with a .22 caliber rifle over a range of muzzle-to-target distances on plywood and MDF panels. Afterward, the depth of the corresponding bullet holes was measured, and the physical damage patterns caused by the bullets were analyzed.

The results show that in plywood and MDF substrates, as muzzle-to-target distance increases, bullet hole depth decreases. This trend was steeper in the plywood substrates than MDF, and bullets penetrated deeper into plywood as well. In addition, this study observed that specific damage patterns to the back of the bullet hole can give hints about the shooter's distance and the bullet's velocity. A predictions model was also developed, using a mathematical formula that relates certain variables to bullet hole depth. Using the data from the test fires, the model was able to predict depth given the substrate material, muzzle-to-target distance, and bullet speed. A visual model that relates the depth of the bullet hole to the muzzle-to-target distance was also generated. Muzzle-to-target distance can be extrapolated from this model, as long as the bullet hole depth is measured and the firearm, cartridge, and substrate material information is obtained from the scene.

Not only does this new method cover the limitations of the Modified Griess test, but it works in tandem with other ballistics analysis methods as well. The procedure is easy to learn and perform, and other tests such as impact angle analysis can be performed without interfering with the bullet hole depth, as it is a non-destructive procedure. Test fires can be performed at the same time with tests for GSR patterns. In conclusion, with some fine tuning, this model may become another useful tool that forensic scientists have available to them at the crime scene.

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### Muzzle-to-Target Distance, Bullet Hole Depth, Wood Substrates

## B111 The Interpretation of Gunshot Residues (GSR) at the Activity Level: State of Knowledge

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**Learning Overview:** The goal of this presentation is to share the state of knowledge on the GSR transfer traces at the activity level as stored in the Transfer Traces at the Activity level Database (TTADB), available to any forensic scientist or legal professional. This research helped to establish the current knowledge, identify gaps in the literature, and therefore suggest future research to be undertaken.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners, forensic scientists, and lawyers access to a user-friendly structured database that inventories the state of knowledge on GSR interpretation in the activity level. This database contains studies on transfer traces at the activity level focusing on transfer, persistence, background, population, and detection of GSR in various scenarios and on different supports that were analyzed, with a critical assessment in a Canadian context.

The purpose of this research, which is part of a larger project on the analysis of studies on transfer traces at the activity level, is therefore to inventory the state of knowledge on GSR interpretation in order to build a structured knowledge database for practitioners and researchers, the TTADB. This database helps identify gaps in the literature and propose lines for incoming research to fill them. The part of the project presented here focuses on GSRs.

GSRs-related papers such as articles, theses, and others (e.g., research reports) were analyzed in order to evaluate the state of knowledge. An analysis of each paper was done to bring out the following characteristics (when possible): year of publication, author(s), country where the study was conducted, type of GSR (organic, inorganic), experimental conditions, and type of study (transfer, persistence, detection, background, population, probabilistic model, methods, etc.). A critical analysis of the relevance of each study regarding the Canadian environment has also been conducted. Once this analysis was completed, the data collected was structured in a database open to forensic practitioners, scientists, and lawyers.

To date, 213 GSR studies, from 1965 to 2020, have been included in the database. The available literature has shown that most of the studies were conducted in various European countries and the United States and very few in Canada. In addition, more studies are conducted on Inorganic GSR (IGSR) than on Organic GSR (OGSR). However, since the advent of so-called non-toxic ammunition, the number of studies on OGSR has tended to increase. Several studies have also recently been carried out on IGSR and OGSR in a complementary manner with a view to finding a viable sequence of analysis and/or comparing the results of the two methods.

Moreover, recent studies have more generally been conducted using high-performance analytical instruments in order to obtain the most sensitive methods of detection rather than focus on issues related to GSR activity and interpretation. However, while it is true that an increase in sensitivity will give greater information on the source of the trace, it will not provide any information as to its relevance, which can only be estimated by considering an interpretation at the activity level. Therefore, techniques such as chromophoric development processes that allow inferences about the shooter, a beholder, and/or a bystander may prove to be fundamental and should be pursued further. Unfortunately, the literature review shows that such research, as well as research incorporating probabilistic or static assessment at the activity level, are rare or even left out.

The purpose of building such a database is to provide a friendly user tool with a large body of literature to assist practitioners in interpreting the data/traces at the activity level. Although the project began with a greater focus on analysis in a Canadian context, international interest in the TTADB is now being expressed.

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### Gunshot Residues, Interpretation, Activity Level





## **B112 An Investigation Into the Source of Contamination of Gunshot Residue (GSR) Primer Found in an Exam Room Used to Analyze Clothing for GSR**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of one way to perform a root cause analysis and preventive action for a GSR contamination incident in the laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners insight into the procedures used to determine the cause and remediation of a GSR contamination.

The laboratory will perform gunshot primer residue testing on GSR stubs from a person's hands when the time from the shooting to the stubbing of a person's hands is less than four hours. In the event that the police do not apprehend the suspect within four hours, the laboratory will perform GSR testing on the clothing the suspect was wearing when the shooting occurred.

The laboratory performs the clothing stubbing in one of the trace evidence laboratory exam rooms. These rooms are thoroughly cleaned once a month to eliminate any possibility of GSR from previous cases contaminating the exam room. In addition, when a case is examined for GSR, the exam tables are cleaned with bleach and a clean piece of white paper is placed on the table. This white paper is stubbed with a GSR stub for a paper blank. The evidence is transferred from the table where it is opened to a different table where the blank white paper is. After the evidence is stubbed, the paper blank is analyzed along with the stubs taken from the clothing. In the 15+ years that the laboratory has performed this procedure, the paper blanks have always been negative.

In April of 2020, a characteristic GSR particle was identified and confirmed on one of the paper blanks.

An investigation was initiated into the source of the particle. The laboratory surmised that there were three possible main causes and several other remote causes for the contamination: (1) the particle came from inadequate cleaning techniques, (2) the particle came from the evidence packaging, or (3) the particle was airborne in the room and landed on the paper. After multiple cleaning and testing of various items in the room, the most plausible source of the GSR particle was from evidence packaging.

As part of the remediation procedure, the following cleaning steps were implemented. The exam room will be cleaned by wiping down the cabinets and exam tables. The wipes will be stubbed and analyzed. If the stubs are positive for GSR, then the procedure is repeated until the stubs come back negative. Items of clothing will be removed from evidence packaging outside the room from where the clothing is processed for GSR. Another room has been designated for processing evidence items known to have GSR on them. Clothing being processed for GSR will not be examined in the above room.

This research takes a novel approach to investigating a GSR contamination issue in the laboratory's exam rooms.

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### **Gunshot Residue (GSR), Contamination, Remediation**

## B113 A Novel Machine Learning Approach Based on Quantitative Profile-Profile Relationship (QPPR) to Address Complex Source-Level Problems in the Forensic Analysis of Gunshot Residue (GSR)

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**Learning Overview:** The goal of this presentation is to describe a novel, machine learning approach to enhance the forensic profiling of organic GSR traces as well as to present its potential to address the most complex source-level problems in the reconstruction of gun crimes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing a new, ground-breaking method for *in silico* profiling of chemical residues that could potentially also be extended to other forensic fields routinely encountering mutable traces.

GSR is a chemical trace that commonly assists law enforcement authorities in the investigation of gun crimes, for example, to recognize whether a suspect has recently been involved in a shooting activity or to estimate shooting distances during ballistic reconstructions. While its use at source level would be extremely useful too (e.g., for origin attribution and/or evidence association), nonetheless, this is still very challenging and limited to specific scenarios. The main problem here is the complexity of the mechanisms involved in GSR formation. In order to advance from this situation and promote a more systematic use of this evidence type, a novel *in silico* approach, QPPR has been developed and is presented herein.<sup>1</sup> This is based on the application of modern machine learning techniques to model the discharge process and predict the pre-discharge chemical profiles of selected ammunition components from those of the respective post-discharge GSR. The obtained profiles can then be compared with another and/or with other measured profiles in order to associate samples and make evidential links during crime investigation.

In particular, the approach was optimized and successfully tested for the prediction of Gas Chromatography/Mass Spectrometry (GC/MS) profiles of smokeless powders from those of the respective organic GSRs.<sup>2,3</sup> Results showed a high degree of similarity between predicted and experimentally measured profiles (media correlation = 0.982) after adequate combination of 14 machine learning techniques, including artificial neural networks and support vector machines. Evaluation of association performances by Receiver Operator Curve (ROC) analysis, for the comparisons between predicted-predicted and predicted-measured profiles, showed classification accuracies of 92.9% and 80.0%, respectively, in extrapolation mode and of 91.7% and 88.3%, respectively, in interpolation mode. These values were close to those obtained after direct comparison of the measured smokeless powder profiles with one another (accuracy = 98.6%), demonstrating excellent potential to correctly associate same-source samples in a number of different forensic scenarios and help to address source-level problems in complex situations. The novel modeling approach is therefore very promising and could unlock wholly new possibilities in the investigation of gun crimes, such as the ability to compare GSRs recovered from different crime scenes and link them in an “intelligence-led” perspective. The benefits are countless and may even extend to the analysis of other GSR fractions (e.g., primer GSR) or to other forensic fields that also routinely encounter mutable chemical traces such as, for example, the analysis of arson accelerants and environmental contaminants.

### Reference(s):

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2. Matteo Davide Gallidabino, Francesco Saverio Romolo, Kelsey Bylenga, and Céline Weyermann. Development of a novel headspace sorptive extraction method to study the ageing of volatile compounds in spent handgun cartridge. *Analytical Chemistry* 86 (2014): 4471-4478, <https://doi.org/10.1021/ac5004568>.
3. Matteo Davide Gallidabino, Francesco Saverio Romolo, and Céline Weyermann. Characterization of volatile organic gunshot residues in fired handgun cartridges by headspace sorptive extraction. *Analytical and Bioanalytical Chemistry* 407 (2015): 7123-7134, <https://doi.org/10.1007/s00216-015-8874-0>.

### Gunshot Residue, Profiling, Chemometrics

## B114 The Reconstruction of Obliterated Serial Numbers in Polymers Using Raman Spectroscopy

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**Learning Overview:** The goal of this presentation is to increase awareness about the existing and the potential non-destructive methods for reconstructing obliterated serial numbers in polymers by touching on acquisition times, system requirements, and detection limits.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a new, promising technique that may allow for this analysis by portable spectrometers, leading to feasible deployment to multiple forensic laboratories and possibly police services.

Forensic practitioners (e.g., ballistic experts) commonly encounter restoration cases of serial numbers, as they provide valuable information for investigative purposes (i.e., identification or individualization of a given object). These numbers are often introduced by stamping techniques. However, criminal activity generally leads to tampering of their depth profile by abrasion in order to prevent reading.

Although some reconstruction techniques already exist in literature for these specific types of materials, they are not effective and are most of all destructive (e.g., require the use of strong acids). Therefore, they are problematic when dealing with a sample that may be the subject of a criminal investigation or trial. In the absence of an efficient and reproducible recovery method, the research group has established a non-destructive laser scanning microscopy scheme to exploit the vibrational Raman spectrum of polymers in order to image strain on different length scales.

This technique, based on the inelastic scattering of photons and optical phonons, has enabled the exploitation of the variations of the peak shift, full width at half maximum (phonon lifetime and local ordering of the material), peak intensity ratio, as well as the correlation of these independent signals.<sup>1,2</sup> As they provide information on the vibration modes of a given molecular bond, these signals can be used to create contrast when imaging a sample that has sustained partial strain (e.g., impression depth of 150µm with a strain depth profile of 750–800µm), despite being obliterated (e.g., obliteration depth of 200µm).

Additionally, recent efforts have been focused on a new signal, the local depolarization ratio. It is defined as the intensity ratio of the spectral band measured, respectively, along the perpendicular and the parallel axis in regard to the direction of polarization of the incident beam. As this ratio provides information on the particular symmetry of vibration modes, it can potentially be used as an imaging tool with promising results in regard to faster acquisition times and data treatment as well as reduced system requirements.

### Reference(s):

1. Parisien Cédric, Gitanjali Kolhatkar, Frank Crispino, André Lajeunesse and Andreas Ruediger. Reconstruction of Obliterated Characters in Polycarbonate through Spectral Imaging. *Analytical Chemistry* 89 (2017): 11648-11652. <https://doi.org/10.1021/acs.analchem.7b03069>.
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### Serial Numbers, Polymers, Raman Spectroscopy

## **B115 Odor Stimuli Validation in Smokeless Powders: The Bridge Between Analytical Chemistry Approaches and Dynamic Airflow Sampling**

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**Learning Overview:** After attending this presentation, attendees will better understand how the use of automated olfactometers for odor stimuli presentation in canine detection applications can be verified with analytical chemistry techniques to provide a laboratory foundation as to the chemical composition in real time. This novel approach allows a concurrent quantitative evaluation of presented odor stimuli.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating not only the feasibility of presenting target explosive odorants using an olfactometer in olfaction exercises, but bridges instrumental validation for confirmation to understand odor chemical composition and survivability concept.

The use of biological detection is crucial within the fields of security screening and criminal investigations. Military and law enforcement personnel utilize canine teams in a range of different applications. Canine disciplines of crucial importance for homeland security purposes include that of explosive and narcotics detection. Due to the ever-changing explosive and narcotic materials encountered during routine field operations, it is imperative to have an optimal training regimen reflective of current target odor needs. Hence, the chemical understanding of target odorant concentrations and subsequent means of odor delivery are essential in the training of a canine team. There are a variety of methods available for the presentation of odor stimuli in olfactory-based exercises, with the use of dynamic airflow sampling being one common method via olfactometers to measure behavioral responses. Concurrently, the chemical characterization of explosive and narcotic materials is an excellent tool to provide valuable information that can not only better inform canine training but can also be used for field-portable technique development.

This study focuses on the use of an in-house olfactometer to test a double-based smokeless powder as a target odor stimulus. Previous work has identified derivatives of smokeless powder additives to include diphenylamine that are formed as the powder material degrades. Using this target odor volatile for study, an instrumental analysis evaluation has been performed to test target volatile after dynamic airflow sampling. Instrumental parameter optimization included analysis of optimal solid phase microextraction fiber chemistry, amount, and age of target odorant as a function of peak area response using Gas Chromatography/Mass Spectrometry (GC/MS). Studies were conducted directly over the headspace of the target odorant and using the olfactometer as the dynamic airflow device for comparison purposes. Previously established volatile organic compounds from smokeless powders were detected, and comparison between non-airflow versus airflow sampling was achieved.

The need for enhanced olfactory-based behavioral tools is essential for optimal applications of biological detectors. This study evaluates not only the feasibility of presenting target explosive odorants using an olfactometer in olfaction exercises but bridges instrumental validation for confirmation to understand odor chemical composition and survivability concepts.

*This study was funded in part by the Department of Homeland Security Science & Technology Directorate (Contract 70RSAT20CB000010).*

**Explosive Odorants, Olfactometer, Analytical Chemistry**

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## **B116 The International Close Non-Match Library (ICNML): An International Known Source Database for Friction Ridge Training, Testing, and Research**

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**Learning Overview:** After attending this presentation, attendees will be aware of an ongoing project to develop a fingerprint database, called ICNML, with known ground truth samples and close non-match exemplars that will be made available to trusted law enforcement agency partners for testing, training, and research. Attendees will also understand some of the benefits and pitfalls of using international Law Enforcement Agency sourcing (LEAsourcing) to distribute tasks on a large project.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a valuable tool for testing, training, and research and information about a new way of engaging a wide range of partners to share the load of large projects. Special attention will be paid to the IT infrastructure put in place to give donors the required guaranties in terms of privacy (by design), rights to revocation, data security, and traceability.

The friction ridge community has a need for known-source comparison materials that can be used in creating competency tests and training samples, as well as for use in research. Unfortunately, the creation of these materials is extremely time- and labor-intensive and most laboratories that have such samples retain them for internal use. For a time, the National Institute of Standards and Technology's Special Database 27 (NIST's SD27) was a popular source of samples, particularly for use in research. However, privacy concerns have resulted in that database being removed from public domain. Additionally, the paired impressions in SD27 were determined to be mated by a consensus of experts but were not "ground truth" samples that had been collected under controlled conditions.

This presentation reports on an ongoing project funded by the National Institute of Justice to create a known source friction ridge database for the use of the law enforcement community using an LEAsourcing strategy. In addition to the provision of known-source comparison samples, a great need of the friction ridge community is challenging samples from "close non-matches" that allow for high difficulty testing and training and also allow trainees to specifically be trained in how to recognize close non-match situations and avoid false identifications. This project is focused on the generation of known close non-match exemplars to include in the ICNML repository. Individuals with access to the database can specify criteria to build testing and training packets, including number of same- and different-source trials and number of easy and close non-match different-source exemplars. Both finger and palm impressions are included in the database.

This presentation will include a live demonstration of ICNML, including showing its main contents, how testing and training packets can be built and downloaded, and reviewing the security measures in place to protect the identities of the individuals whose impressions are stored in the database. This presentation will also discuss who will be granted access to the completed database and what the allowable uses will be. Access to the database will be free to all authorized parties.

Creating a large database that is populated with known source impressions from many individuals and including close non-matches is not a small undertaking. The creation of marks and exemplars, selection of search areas, and searching of large databases to locate close non-matches is work that requires many hours of labor as well as touching on regulations and policies that can vary widely from jurisdiction to jurisdiction. For that reason, this study hit upon an international LEAsourcing model whereby the tasks have been parsed out in small chunks to many project partners located in laboratories around the world. In this way, the workload is divided as well as allowing for sensitive work to be performed in jurisdictions where regulations allow it. Part of this presentation will address the structure put in place to accomplish this, the benefits of such an approach, and challenges faced in utilizing international LEAsourcing. The hope is that this description will help other researchers who may be considering utilizing similar approaches to large-scale projects.

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### **Fingerprints, Known Source Collection, Close Non-Match**

## B117 A Field Analysis of Laboratory Case Processing: Latent Print Comparison and Examiner Conclusions

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**Learning Overview:** The goal of this presentation is to educate attendees about typical real-world procedures and conclusions spanning one calendar year within a large latent print comparison unit. This presentation will also explore sources of variability in sufficiency and identification conclusions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding upon a very limited research base and discussing how results can shape future research, policy, and professional practice. Attendees will be encouraged to introspect upon common practices within their own and their laboratory's casework.

Research examining the efficacy and reliability of latent print comparison has expanded in recent years in response to scholars highlighting the need for additional empirical support for many forensic science disciplines and calling attention to potential contextual effects in analytic conclusions.<sup>1,2</sup> A small body of research has attempted to elucidate the error rates of latent print comparison as a forensic discipline, and a growing body of research has examined the influence of contextual effects upon latent print comparison, suggesting that a number of task-irrelevant factors can influence conclusions.<sup>3-7</sup> However, almost no research has examined actual latent print casework to first determine typical analysis procedures and outcomes. Beyond the potential influence of contextual effects, broad examination of actual laboratory case processing is sorely needed, yet lacking.

Per research, there has been only one study of real-world outcomes in latent print comparison.<sup>8</sup> The current study sought to expand upon that study by achieving the following: (1) describe the casework completed by latent comparison examiners in a large laboratory over the course of one calendar year (i.e., 2018); (2) describe the prevalence of examiner conclusions during one year; (3) explore whether examiner conclusions vary according to casework variables such as latent print type, offense type, or Automated Fingerprint Identification System (AFIS) system use; and (4) explore the extent to which there are examiner differences in examiner conclusions and case processing. Researchers examined all latent print cases with reports dated 2018 within a large crime laboratory in Texas. In total, 17 latent print examiners submitted reports in 2018. All examiners were certified by the International Association for Identification, and work experience ranged from 5 to 36 years.

This presentation will provide detailed charts and statistics summarizing requests for latent print comparison and examiners' subsequent conclusions during 2018. In brief, the latent print unit addressed 3,239 analysis requests relating to 2,975 cases in 2018. Of the cases, 23.7% were person offenses. Of the 20,494 individual prints examined in 2018, 44.8% were deemed to be of sufficient quality to enter into AFIS. Few prints (1.7%) were deemed to have comparative value but be of insufficient quality to enter into AFIS. Slightly more than half (53.5%) of all prints were determined to have no comparative value.

Houston Forensic Science Center (HFSC) research examiners conducted 11,812 AFIS searches during 2018. Most searches were conducted at the county level (65.0%). State-level (16.9%) and federal-level (18.1%) AFIS searches were equally common. Most AFIS searches did not result in potential identifications (77.8%). Indeed, only 22.2% of AFIS searches resulted in potential identifications. As will be shown in a flowchart, 12.7% of all examined prints resulted in potential identifications.

This presentation will also describe the variability within sufficiency determinations and AFIS outcomes. For example, print type was significantly associated with sufficiency determinations (i.e., prints deemed to be of insufficient quality for AFIS entry were 2.78 times more likely to be unspecified impressions). Finally, this presentation will detail individual differences among 14 latent print examiners. For example, examiners completed between 12 and 46 requests each month, examining between 66 and 269 prints. Some examiners opined that one of every three examined prints (35.8%) were of sufficient quality for AFIS entry whereas others opined that 56.5% were of sufficient quality. Moreover, some examiners concluded preliminary AFIS associations for 13.3% of entered prints whereas others were two times as likely (27.1%) to conclude that an AFIS association existed.

Taken together, the current findings are among the first to describe typical, real-world casework completed by a latent print comparison unit. Results suggest variability in examiner conclusions that may be partially explained by AFIS system, print type, offense type, and individual differences. Given the lack of research examining influences upon real-world latent print conclusions, it is critical to continue examinations of crime laboratory casework and determine the extent to which external information may be associated with conclusions.

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### Latent Prints, Crime Laboratory, Case Processing

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## B118 Implementing a Testimony Transcript Review Process

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**Learning Overview:** After attending this presentation, attendees will have gained insight into how to implement a testimony transcript review program. Attendees will learn how the program was developed, how transcripts are received and evaluated, and how information obtained from the evaluation process is used to drive improvements within the laboratory.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by providing an example of how the Houston Forensic Science Center (HFSC) developed and implemented a robust testimony transcript review program. This presentation will provide information on how reviews of testimony transcripts provide an additional means of monitoring employee performance and improving courtroom testimony by identifying opportunities for improvement.

HFSC implemented its transcript review program in March of 2018. The concept for the program grew out of a desire to improve HFSC's testimony monitoring program by providing feedback from different perspectives and to create a blind testimony monitoring program. HFSC's objective for the program is to identify opportunities for improvement and to ensure that testimony by HFSC forensic practitioners is provided to our criminal justice system in a clear, concise, and accurate manner.

HFSC management met with the Harris County District Attorney's Office and the Harris County Public Defender's Office to develop a process for obtaining transcripts for cases sent to the appellate court. The process for reviewing transcripts involves forming a three-person committee comprised of a lay person with no or limited knowledge of the forensic science, a technical expert, and a member of the Quality Division. Each committee member reviews the transcript independently. The committee then meets to review everyone's comments and select the comments they feel will provide the greatest benefit to the forensic practitioner's development as an expert witness. The committee submits a final consensus-based evaluation to the Quality Division. After a final review by the Quality Division, the evaluation is provided to section management or the technical expert for discussion with the forensic practitioner.

Since its initial conception, several improvements to the program have been implemented. The program has been made blind to the committee members by redacting all identifying information from transcripts prior to the committee's review. Another change was to require the forensic practitioner who testified to complete a self-evaluation prior to the transcript being sent to the committee. This evaluation is not provided to the committee prior to their review. Forms used to document the evaluations have been updated over the past two years to allow committee members to better assess the testimony and document opportunities for improvement. Lastly, the program team now includes the legal counsel, who helps identify cases where a forensic practitioner testified, and the case was subsequently appealed.

Within the first two years of implementing the program, two opportunities for improvement were identified. The first was the need to provide training regarding qualifying questions. HFSC presents a unique challenge to staff because it is a local government corporation. HFSC also operates in Texas, which requires forensic laboratories with Firearms, Forensic Biology, Toxicology, and Seized Drugs sections to be accredited by the Texas Forensic Science Commission (TFSC). Additionally, forensic practitioners in those sections are required to be licensed by TFSC. The second opportunity for improvement was ensuring staff understand the difference between expert testimony and lay testimony. These areas of improvement were addressed through training provided to all forensic practitioners. One training session focused on qualifying questions, and another focused on recognizing the difference between expert and lay testimony.

One limitation of the program is that some staff at HFSC do not have many opportunities to testify in court. This has been especially true of 2020, when the Harris County Court system shut down because of the COVID-19 pandemic. Review of future transcripts will provide HFSC with the ability to evaluate the effectiveness of the training provided in response to the program.

HFSC's process for transcript reviews was successfully implemented and improved during the first two years of operation. The program has also led to the identification of opportunities for improvements regarding courtroom testimony. The transcript review program provides a broader perspective for monitoring courtroom testimony that helps to ensure forensic practitioners continue to communicate effectively while testifying in courts of law.

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### Testimony Transcript Review, Blind Transcript Review, Monitoring Testimony

## B119 Clarifying Blood Pool Vocabulary

Rachel A. Johnson, MS\*, Chesapeake Police Department, Chesapeake, VA 23322

**Learning Overview:** After attending this presentation, attendees will understand the necessity for appropriate definitions of blood pools applicable to crime scenes and court testimony.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the need for standardized contextual definitions of blood pools.

The language of science is essential to accurately conduct and report scientific findings. Correct standardized blood pool terminology is imperative to avoid uncertainty in evidence. An overview of relevant literature is provided. Current definitions associated with “blood pools” are vague, focusing on biological and physiological processes. Revision of relevant vocabulary is necessary for standardization and interpretation of each chronological coagulation phase. Chronology is then applied to crime scene reconstruction, relating to time of injury.

The American Standards Board definitions regarding “blood clot,” “serum stain,” and “blood pool” are problematic. Literature generally provides biological information, not visual criteria for characteristics such as coagulation chronology.<sup>1</sup> Without standards for the initiation of each coagulation phase, this information cannot be used to determine time of injury at a scene.

“Blood clots” are described as a gelatinous mass formed through complex biological processes.<sup>1</sup> Some literature’s benchmark for clot formation is “the point at which no flowback occurs.”<sup>2</sup> Other literature used “no flowback” for determining the initiation of a clot.<sup>3</sup> However, “no flowback” as a standard is subjective and ambiguous, leading to potential error. Confusingly, literature records the beginning of clot formation to be between 5 and 20 minutes, and pool spread, implying a lack of clot completion, to continue for 15 to 30 minutes.<sup>2,4</sup> Clear and concise definitions allow interpretation of initial clot formation.

A “serum stain” is the liquid portion of blood separating from the solid portion as a clot is forming.<sup>1</sup> Literature neglects a standard for the initiation of serum separation. Serum stains do not form in “smaller pools,” an arbitrary description of blood volume.<sup>4,5</sup> A benchmark is necessary for determining the commencement of a serum stain, otherwise the exact volume of “smaller pools” cannot be ascertained. Serum separation is also an indicator of clot contraction, another important marker in the chronology of coagulation.

“Blood pools” are currently defined as an accumulation of blood on a surface.<sup>1</sup> This definition is similar to a “blood stain”—a deposit of blood on a surface.<sup>1</sup> “Blood pools” and “blood stains,” however, are different. “Blood stain” is generically used for deposited blood at a crime scene. Therefore, contextual markers are indispensable to distinguish a “pool” from a “stain.” These misnomers can be avoided through definitional revision of “blood clot” and “serum stain.” For example, a “blood pool” should be defined to exhibit serum separation, thereby limiting “smaller pools” from the current interpretation. Revised descriptive definitions will provide a clearer interpretation of a crime scene, accurate written reports, and reliable court testimony.

Current definitions of vocabulary regarding blood pools must be revised by a standards board (National Institute of Standards and Technology [NIST], Organization of Scientific Area Committees [OSAC], etc.). Once benchmarks for each phase have been determined, research on the effect of internal and external factors (such as anticoagulants, humidity, volume, etc.) will be applied to crime scene reconstruction. “Blood clots” and “serum stain” terminology require clear standards to determine the initiation of each coagulation phase. With an accurate and reliable interpretation of the chronology of coagulation, a contextual definition of blood pools can be applied to crime scenes. Much like the dissemination of current standardized terminology, a publication should be made available to the relevant disciplines. Seminars and trainings should be conducted to ensure accurate and confident interpretation of corrected standards of the revised definitions.

Standardized scientific terminology creates uniform, non-ambiguous reporting of scientific evidence and testimony. Based on surveyed literature, a revision is needed for the definitions of “blood clot,” “serum stain,” and “blood pool.” While current definitions discuss biological and serological facets of blood pools, they do not address the chronology of coagulation. Appropriate contextual addendums to current definitions should minimize subjective interpretations and confusion concerning time since injury in crime scene reconstruction. These findings will then be accurately and reliably conveyed in written reports and court testimony. Clarification is needed for blood pools in crime scene reconstruction.

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### Vocabulary, Crime Scene, Blood Pool



## **B120 A Validation Study of Applied Biosystems™ VeriFiler™ Plus Polymerase Chain Reaction (PCR) Amplification Kit**

*Jessica M. Esparza, PhD\*, Shreveport, LA 71103*

**Learning Overview:** The goal of this presentation is to introduce attendees to data from the internal validation of a new megaplex PCR amplification kit and the issues encountered prior to the kit's submission to the National DNA Index System (NDIS) for approval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing another megaplex PCR amplification kit that includes internal quality control markers that monitor PCR efficiency, sample quality, and the possible presence of inhibitors on a per-sample basis.

Several megaplex PCR amplification kits exist that include the expanded Combined DNA Index System (CODIS) Core Loci required by the Federal Bureau of Investigation (FBI) as of January 1, 2017. However, only six PCR kits contain internal quality control markers, and only one kit was approved by NDIS before this validation was completed. The Applied Biosystems™ (AB) VeriFiler™ Plus (VFP) PCR Amplification Kit is a new 6-dye Short-Tandem Repeat (STR) multiplex assay for the amplification of human genomic DNA.<sup>1</sup> The VFP PCR kit amplifies 23 autosomal STR loci (including the expanded CODIS Core Loci), two internal quality control markers (IQCS and IQCL), one insertion/deletion marker on the Y chromosome (Y indel), and amelogenin (sex determining marker). The IQCS and IQCL are two synthetic targets, one low molecular weight and one high molecular weight, which are amplified simultaneously with the sample. The internal quality control system evaluates the success of the PCR and indicates sample quality.

The studies performed at the North Louisiana Criminalistics Laboratory (NLCL) were in accordance with the FBI's Quality Assurance Standards for an internal validation. The studies included sensitivity, precision, accuracy, contamination, concordance, known and non-probative samples, and mixture samples. Extracted DNA was quantified using the Quantifiler<sup>®</sup> Trio DNA Quantification Kit. All samples were run on the Applied Biosystems™ 7500 Real-Time PCR System and analyzed using Human Identification (HID) Real-Time Software v1.3. Samples were amplified on GeneAmp™ PCR System 9700 thermal cyclers using two-stage cycling parameters with max ramping for a total of 29 cycles. Amplified product was run on the Applied Biosystem™ 3500 Genetic Analyzer using 3500 Series Data Collection Software 4 (DC v4). GeneMapper™ ID-X Software v1.6 was used for data analysis.

During the validation of the VFP PCR kit, several reproducible and non-specific artifacts were characterized that could not be attributed to the kit or the 3500 Genetic Analyzer. The cause of these artifacts was determined to be the DC v4. Specifically, the Signal Optimization (SO) feature is intended to reduce the peak height variation caused by the instrument optics and injection conditions primarily for 24-capillary instruments. Since the VFP validation was performed on 8-capillary 3500 instruments, it was necessary to complete a performance check with the SO feature disabled. The performance check demonstrated that contamination, sensitivity, precision, and accuracy were not significantly affected with the SO feature disabled. However, the non-specific artifacts observed in the validation data were greatly reduced, which correlated with a cleaner baseline.

The studies in the validation demonstrate the VFP PCR kit is a robust, reliable, and sensitive STR multiplex assay for the amplification of specific loci in human genomic DNA. Sensitivity and linearity are maintained when samples are of ample quantity and are consistent with the developmental validation. Single-source samples show reproducibility of genotype, and the mixture samples cover numerous contributor ratios and sample conditions. Concordance was demonstrated between the current PCR kit and the VFP PCR kit. Precise and accurate results were obtained using the VFP PCR kit with a lack of contamination. Although a number of artifacts were observed in the validation study, the performance check verified the artifacts were attributed to the DC v4 Software and not the VFP PCR kit.

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- <sup>1</sup> ThermoFisher Scientific. VeriFiler™ Plus PCR Amplification Kit User Guide, Revision B.0.

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### **DNA Analysis, STRs, Validation**



## **B121 The Development of a Five-Dye Insertion/Deletion (INDEL) Panel for Ancestry Determination**

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**Learning Overview:** After attending this presentation, attendees will understand the benefits of INDEL and Ancestry Informative Markers (AIMs). This presentation will focus on their application with low-quantity DNA and their ability to provide investigative leads. This research developed an INDEL panel for ancestry prediction followed by a validation according to the Scientific Working Group on DNA Analysis Methods (SWGAM).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel INDEL panel for ancestry prediction. Additionally, the biogeographic prediction power of this panel was demonstrated with degraded and challenging samples.

The use of Short Tandem Repeat (STR) markers to find individualizing features in the human genome in conjunction with the national Combined DNA Index System (CODIS) database has streamlined human identification. DNA evidence continues to evolve as improvements in sensitivity allow for utilization with many forensic samples. As effective as individual identification markers are, other marker types have been shown to be complementary in forensic cases. INDEL markers are similar to STRs in that they provide genetic identification but differ in other ways. INDELs are bi-allelic, which limits human identification, but two important benefits were exploited in this study. INDELs can be genotyped using small amplicons, which allows for greater success with degraded/low quantity sample types. Additionally, ancestry prediction is possible because INDEL markers with high Fst values and low mutation rates can be targeted for analysis.

This research focuses on the use of INDEL polymorphisms that were selected for their ability to differentiate ancestry. These markers, known as AIMs, can distinguish biogeographic differences as these markers exhibit different allele frequencies between populations. Additionally, INDEL amplicons were chosen because they can be designed to be differentiated by size and integrated into a Capillary Electrophoresis (CE) platform. An advantage INDELs have over STRs is the ability to amplify many markers (25–30) using amplicons less than 200bp. INDELs have the added benefit of no stutter artifacts allowing for easier interpretation from degraded samples.

This presentation will discuss the limitations of current individual identification testing and how the developed panel can serve as a complement to forensic cases. The panel consists of 25 ancestry informative INDEL markers configured across five dye channels and validated according to SWGAM guidelines. Stability, mixtures, sensitivity, and reproducibility were evaluated to ensure the effectiveness of the panel. This panel assessed biogeographic separation of samples from African American, Hispanic, Caucasian, and East Asian groups. PCA plots were formed to determine separation of individuals based on self-identified ancestry and INDEL results. In conclusion, a 25 marker INDEL panel was developed, and preliminary results indicate the assay can separate populations based on AIMs and effectively recover full profiles down to 32 pg and partial profiles at 16pg.

### **Insertion Deletion Polymorphisms, Capillary Electrophoresis, Ancestry Informative Markers (AIMs)**

## B122 Human Identification Using the Skin Virome

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential for using viral DNA samples from humans' skin as an alternative genetic marker. This study presents data from research demonstrating that sufficient genetic diversity exists in a portion of the viral meta-populations residing on human skin to create a DNA profile that may be appropriate for forensic identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing some of the different types of viruses that are commonly detected on human skin and the processes required to collect those virome samples and derive patterns from them suitable for comparisons. The relative abundance of viral particles on human skin and the wide range of classification groups, as well as the stability of those characteristics, will be shown. This study will offer the forensic community a new tool to potentially identify biological samples when human DNA is not present in usable quantities.

The human bacterial microbiome has already been examined as an alternative method for postmortem interval determination and as a biological marker in cases involving soil samples. The human virome offers additional advantages, as viral genomes are even smaller than those of bacteria and thus are potentially more stable. They also have a variety of transcription strategies (for example, double- and single-stranded), increasing the possible number of discriminating markers; and are present throughout the human body, including the skin and body fluids, making them transferrable. The copy number of viral genomes in a given volume is substantially higher as well, compared to the copy number of human or bacterial genomes, increasing the likelihood of isolating a sufficient quantity for successful testing.

This study recruited 60 adult subjects (25 male and 35 female) for sample collections, including two sets of co-habiting couples. Direct samples of the skin virome were collected from each subject at five time points over the course of six months (baseline, two weeks, one month, three months, and six months) from each of their hands and their scalp. A pipeline was developed for viral isolation, amplification, and sequencing. This method used the inherent small size of viral particles to remove bacteria and other large cells (fungi, human, etc.) away from the viral particles by filtering with a 0.2 micron filter. The resulting viral particles were lysed and the DNA from viral particles extracted using the QIAamp<sup>®</sup> MinElute Virus Spin Kit. The resulting DNA was then amplified using Multiple Displacement Amplification (MDA) with the Sygnis TruePrime<sup>®</sup> WGA Kit and associated protocol to increase viral DNA yields. The amplified DNA was used for library preparation and sequencing on the Illumina<sup>®</sup> HiSeq platform. Library barcoding and preparation was performed using New England Biolab's NEBNext Ultra II library preparation kit. In addition to the virome samples collected from each study participant, metadata was collected with each sampling regarding use of hand sanitizers, hand and hair washing intervals, travel, and contact with domestic animals.

Quantitation of viral DNA and sequencing results show that the collection and preparation protocols developed in this study provided large amounts of testable virome DNA. Samples from every person and time point have been successfully processed, while human and bacterial sequences were filtered out, ensuring data quality. Analysis of the sequence information showed that virome patterns of specific viral groups can be clearly and easily differentiated among the study subjects, the majority of the virome pattern is the same between the different body parts, and each persons' virome pattern of these groups of interest did not demonstrate substantial changes between the first collection and the six-month collection.

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### Virome, DNA, Human Identification

## B123 I-FAMILIA: An Innovative Service Offering International DNA Kinship Matching Capacity for Missing Persons Identifications

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**Learning Overview:** The goal of this presentation is to present the innovative service INTERPOL Family Associated Matching to Identify Lost Individuals Abroad (I-FAMILIA), which will bring an international DNA kinship-matching capacity to all member countries for their missing persons investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping ongoing and future missing persons investigations by facilitating international DNA kinship matching and data exchange between police worldwide

In light of the ease of international travel, an increased global migration, and the consequences of international crime and human trafficking that results in a vulnerability of migrants and refugees, many Missing Persons (MPs) investigations exhibit a cross-border dimension. When national MP DNA programs have exhausted all possible leads, an international DNA comparison needs to be adequately considered.

The International Criminal Police Organization (INTERPOL), is the world's largest international police organization, connecting 194 member countries and provides the legal framework and technical infrastructure for a secure exchange of police information. INTERPOL's international DNA sharing platform provides a structured means for member countries to send antemortem DNA profiles (conventionally obtained from personal items of the missing person) and postmortem DNA profiles (obtained from Unidentified Human Remains [UHR]) for an automated comparison in the INTERPOL DNA database against data previously entered by member countries.

In many cases, antemortem DNA profiles from MPs are either unavailable or of insufficient quality to confirm the identity of the MP. Consequently, antemortem DNA data can only be obtained through biological relatives and the fit of an MP for any given pedigree (e.g., parent, child, sibling of the MP) is estimated by the computation of Likelihood Ratios (LR) using specific allele frequencies from the reference population.<sup>1</sup> Although kinship DNA matching is relatively easy to implement nationally, where the MP and UHR are reported within the same country, many challenges need to be resolved before applying this method to an international configuration. First, ancestry is very often unknown or inaccurately reported and the use of allele frequencies from the wrong reference population could lead to erroneous conclusions as rare alleles in one population may not be as rare in another population. Second, while kinship DNA analysis is performing well with full DNA profiles, many DNA profiles are partials, due to the nature of degraded DNA from UHR samples. Also, the heterogeneity of analytical Short Tandem Repeat (STR) kits used by different laboratories leads to a reduced number of common STR markers and can give rise to weak LR values. Third, arbitrary LR thresholds are often used to determine whether or not a match should be reported but are not accurate for several types of pedigree and/or partial DNA matches.

Considering that international DNA kinship matching would positively impact international police cooperation for MP investigations, INTERPOL has launched I-FAMILIA, an innovative service aiming to facilitate and standardize the international DNA sharing and comparison of UHRs, MPs, and their relatives' profiles.

I-FAMILIA consists of three components: a dedicated database to host the anonymized DNA profiles from the biological relatives of reported missing persons; the DNA matching software, called Bonaparte™, developed by SMART Research BV to perform kinship calculations; and new accompanying interpretation guidelines to efficiently identify and report potential matches between UHR samples and family pedigrees in an international context.<sup>2</sup>

The developmental validation of the service was based on LR calculations using worldwide allele frequencies, allowing kinship analysis in an ancestry-neutral manner.<sup>3</sup> Tailor-made determination of optimal LR thresholds, based on data simulation of related and unrelated pedigrees, were calculated for nine common scenarios found in MP investigations and for a common number of STR markers between 7 and 24 to mimic partial DNA profiles often transmitted by member countries. Streamlined interpretation tables are then used to interpret the LR values for each specific case to make an informed decision of rejection or confirmation of the potential association. In case of a positive hit, a potential match report will be sent to both countries who will then be able to compare, in accordance with their national procedures, antemortem data and postmortem data by using additional means of identification to officially identify the MP and ultimately bring closure to families expecting news of their loved ones.

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### Missing Person, Kinship, International

## B124 Proteomic Genotyping: Increased Sensitivity Using Targeted Mass Spectrometry Platforms

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**Learning Overview:** This presentation will provide attendees with information regarding the efficacy and sensitivity of proteomic genotyping. Attendees will learn that there are other genetically identifying information types that are available to investigators when DNA-based information is partial, incomplete, or missing from an evidentiary sample. Attendees will learn more about this novel genotyping approach, where it would be useful to investigators, and the potential for this method in extracting genetic information from difficult samples. Attendees will also learn about the potential of forensic proteomics.

**Impact on the Forensic Science Community:** Protein is a component of all biological evidence. Protein is more stable than DNA and recent advances in proteomic mass spectrometry allows protein to be analyzed with increasing depth and precision. Protein also carries genetic information in the form of genetically variant peptides. This information is novel and can be accessed when DNA is compromised or degraded. In this way investigators may be able to increase the discriminatory power of degraded evidentiary samples.

Proteomic genotyping is a mass spectrometry-based method that infers the genotype of non-synonymous Single Nucleotide Polymorphism (SNP) alleles by detecting the resulting single amino acid polymorphisms found in genetically variant peptides in proteomic datasets from evidentiary samples. Like any genotype, these can be used to statistically associate an individual to forensic evidence. The utility of the inferred genotype increases as the detection of genetically variant peptides increases and as the technology is transferred to mass spectrometry platforms available to forensic practitioners.

Digests of single (2cm) human hair shafts from three European and two African subjects were analyzed using data-dependent acquisition on a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ system, data independent acquisition and a variant of parallel reaction monitoring on a Orbitrap Fusion™ Lumos™ Tribrid™ system, and multiple reaction monitoring on an Agilent® 6495 triple quadrupole system. Average genetically variant peptide detection from a selected 24 genetically variant peptide panel increased from  $6.5 \pm 1.1$  and  $3.1 \pm 0.8$  using data dependent and independent acquisition to  $9.5 \pm 0.7$  and  $11.7 \pm 1.7$  using parallel reaction and multiple reaction monitoring ( $p < 0.05$ ). Targeted methods of analysis resulted in a 1.3-fold to 1.6-fold increase in detection sensitivity compared to the standard shotgun proteomic methodology. This increase in biomarker detection has a functional impact on the statistical association of a protein sample and an individual. Increased biomarker sensitivity, using Markov Chain Monte Carlo modeling, produced a median estimated random match probability of over 1 in 10 billion for parallel reaction monitoring methodologies and 1 in 10 trillion for multiple reaction monitoring methodologies from a single hair using targeted proteomics. Detected genetically variant peptides were validated by the inclusion of stable isotope labeled peptides in each sample as well as independent confirmation of inferred genotypes by direct exome sequencing.

This research accomplishes two aims: the demonstration of utility for alternative analytical platforms in proteomic genotyping and the establishment of validation methods for the evaluation of inferred genotypes by forensic practitioners. Importantly, the targeted proteomic platform of multiple reaction monitoring is readily available to forensic toxicologists and investigators.

### Proteomic Genotyping, Genetically Variant Peptides, Forensic Proteomics

## B125 Body Fluid and DNA Persistence on Juvenile Victims of Sexual Assault

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the persistence of body fluid and DNA on juvenile victims of sexual assault.

**Impact on the Forensic Science Community:** This presentation will directly impact the forensic science community by seeking to provide a scientific basis for changes to state and national protocols for evidence collection from juvenile victims of sexual assault. Additionally, practitioners who attend the presentation will gain knowledge that can directly inform procedures within their laboratory as well as their own testimony.

The primary goals of this study are to evaluate body fluid and DNA persistence on juvenile victims of sexual assault, inform state and national protocols for evidence collection, and inform testing protocols within forensic laboratories. Previous studies have evaluated body fluid and DNA persistence from consenting, sexually active adults as well as on living and deceased adult victims of sexual assault. Policies regarding the length of time after an alleged assault to collect various types of samples from adult victims of sexual assault are based on the empirical data presented in such studies.

A National Protocol for Sexual Abuse Medical Forensic Examinations: Pediatric (2016) specifies Sexual Assault Evidence Kit (SAEK) collection “within the prescribed jurisdictional time frame (which should be a minimum window of 72 hours since the sexual abuse)” and that “case circumstances and future research may indicate a need for an acute examination and forensic sample collection beyond that time frame.”<sup>1</sup> The National Protocol recommends the involvement of “pediatric examiners and crime lab personnel in determining how any new information results in changes in practice.”<sup>1</sup> National Best Practices for Sexual Assault Kits (2018) issued by the National Institute of Justice recommends sample collection “up to five days or longer post-assault” and cites advancements in DNA sensitivity for “potential to extend the time frame to nine days post-assault in the living patient.”<sup>2</sup> These statements are echoed in A National Protocol for Sexual Assault Medical Forensic Examinations: Adults/Adolescents (2013).<sup>3</sup> Neither document specifies that the support for increasing the amount of time post-assault for kit collection due to increased sensitivity of DNA testing is only applicable to adult victims.

This collaborative study between a certified Sexual Assault Nurse Examiner and forensic laboratory personnel seeks to provide casework data from over 500 cases of child sexual assault to inform changes to policies pertaining to evidence collection from juvenile victims of sexual assault. The persistence of acid phosphatase, prostate-specific antigen, spermatozoa, and foreign DNA detected with Quantifiler™ Trio DNA Quantification Kit, GlobalFiler™, and YFiler™ Plus will be presented, as applicable. Preliminary findings from this study indicate that foreign DNA can be detected beyond 72 hours in internal and external samples collected from juvenile victims of sexual assault. These preliminary findings indicate that changes to state and national protocols for juvenile victims of sexual assault may be warranted to ensure that probative evidence is collected.

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3. U.S. Department of Justice Office on Violence Against Women. A National Protocol for Sexual Assault Medical Forensic Examinations: Adults/Adolescents.

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### Persistence, Juvenile, Sexual Assault

## B126 Pull-Up Problems: A Method to Better Identify and Characterize Pull-Up Peaks

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**Learning Overview:** After attending this presentation, attendees will understand how to develop and implement a standardized procedure to evaluate pull-up artifacts, allowing them to determine interpretation guidelines to reduce the complexity of electropherogram analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework to develop interpretation guidelines regarding pull-up artifacts in electropherograms.

One of the key steps in DNA analysis is the evaluation of the peaks present in the electropherogram to determine if they are alleles or artifacts. For most of the peaks, alleles will be easily distinguished from artifacts. However, in complex DNA results such as mixtures, this exercise can prove to be more difficult and can be critical for the evaluation of the number of contributors, a crucial step for probabilistic genotyping interpretation. One type of artifact that can cause interpretation issues is the pull-up artifact, which appears at the capillary electrophoresis step, and can be labeled as alleles by the genotyping software. In mixtures, such peaks can be confused as a minor contributor's allele and could create situations of false exclusions.

The goal of this study was to develop a method of evaluation of pull-up artifacts and develop guidelines for the detection of these peaks. To achieve this goal, a plate of 22 pooled Polymerase Chain Reaction (PCR) products from known samples was created, with both single source profiles and mixtures. Capillary electrophoresis injection plates were set up from that PCR plate and the pull-up artifacts were compiled. The PCR product was sourced from positive controls amplified with Identifiler™ Plus, the capillary electrophoresis done on an Applied Biosystems™ 3500xL Genetic Analyzer, data was collected with the Applied Biosystems™ 3500 Series Data Collection Software 3 v3.0, and the analysis done using GeneMapper® ID-X v1.5. A total 36 injections were done over the course of five months, for a total of 792 samples analyzed and evaluated for the presence of pull-up artifacts. The size, height, and dye channel were compiled for the 5,092 pull-up peaks observed and also the alleles causing each of these artifacts. The relative height and the distance of the pull-up to its source were calculated and graphed out, as well as a few other parameters. The data collected was used to determine pull-up patterns from which interpretation guidelines were established. Although these results cannot be directly applied in other laboratories, pull-up artifacts are observed with all PCR-amplified DNA separated on a capillary electrophoresis instrument. Therefore, the presented method of characterization of pull-up artifacts could be implemented in any laboratory, using the amplification kit of choice and specific capillary electrophoresis injection parameters. Until these artifacts are successfully managed or eliminated by software or hardware, the study of pull-up can prove useful to analyze DNA results with increased confidence.

### Pull-Up, Capillary Electrophoresis, DNA Analysis

## B127 The Development of a DNA Extraction Method From Rootless Hair Shafts

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**Learning Overview:** After attending this presentation, attendees will understand the evaluation of a novel extraction and bead purification kit compared to multiple methods for nuclear DNA extraction from rootless hair shafts, demonstrating the potential for the use of this new technology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect of rootless hair extraction as it can augment traditional means of extraction and help to provide further benefit for a class of evidence that is underutilized in the forensic laboratory.

Reported here is a new magnetic bead DNA extraction system for the extraction from highly degraded biological samples. The magnetic beads used were designed to capture degraded DNA, such as found in a hair shaft from rootless hair. The digestion buffers and binding buffers were optimized for the hair samples.

Forensic crime laboratories receive hair shafts and, in particular, rootless hair shafts as evidentiary samples and process them for DNA evidence as a means of identification of individuals. Oftentimes, nuclear DNA is too scarce and degraded to enable sufficient profile recovery from hair shafts using standard methods. Even with current Short Tandem Repeat (STR) kits available on the market, the ability to obtain meaningful profiles may be limited due to the amount of nuclear DNA available to the laboratory. In most cases, laboratories will potentially try and extract mitochondrial DNA (mtDNA) from the hair shafts as there are hundreds of thousands of copies per cell compared to the nuclear DNA. However, the process of recovering, processing, and analyzing the mtDNA can be labor intensive and time consuming on resources in the forensic laboratory. This presentation presents an extraction kit with specially designed buffers and magnetic beads capable of recovering fragmented and low-level DNA from samples such as rootless hair shafts. This will save time and effort on the part of the labs as it fits into the standard workflows of forensic laboratories and has the capabilities of producing greater DNA quantities and more successful STR profiles for difficult sample types.

The studies show results from rootless hair shafts both washed and unwashed using the InnoXtract™ method as well as a comparison to the EZ1™ automated investigator kit. The InnoXtract™ was capable of recovering 0.0015ng/μL compared to the EZ1™ obtaining 0.0006ng/μL of DNA from extraction. Further tests evaluated the length of the rootless hair and the InnoXtract's™ ability to recover larger amounts of DNA from longer pieces of hair strands compared to a traditional method of extraction giving the potential strength to utilizing the InnoXtract™ kit. Further studies looked at artificially degraded sample recovery, mock sample types, and further testing adhering to the Scientific Working Group on DNA Analysis Methods (SWGDM) guidelines.

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### DNA Extraction, Rootless Hair DNA, InnoXtract™



## B128 The Extraction and Genotyping of Human DNA in a Still Body Aqueous Environment

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the degradation and migration of DNA from epidermal tissue in a still body of water. The importance of this study is to help implement new protocols with forensic applications to increase the number of missing person cases solved.

**Impact on the Forensic Science Community:** The use of forensic science is minimal in missing person cases unless unknown remains are found. This presentation will impact the forensic science community by providing information on a DNA processing method that could be added to the current protocols for missing person cases. This study will encourage the involvement of forensic applications early on in the search for missing persons cases and hopefully reduce the time the individual remains are unaccounted for.

The number of missing person cases has increased in the United States alone from 85,459 active cases in 2018 to 87,438 active cases in 2019, but this research attempts to provide a novel forensic technique for solving more of these cases.<sup>1</sup> The presence of DNA, from the deposition of human epidermal tissue, in a still body of water, such as a lake or pond, was sampled every other day at specific intervals over a 30-day period. Sampling occurred every 4 feet starting at the sample and stopping at 12 feet from the sample origin. A total of five samples was taken every other day. This study will determine the length of time source DNA can be detected and if it can successfully be genotyped when collected from an aqueous environment. The study will also determine if weather conditions cause an increase or decrease in the migration or degradation of the DNA being observed. By repeating the experiment for the spring and summer seasons, along with a control sample for each, possible trends can be established for varying weather conditions. The purpose of the control sample is to indicate if there are any interactions within the aqueous environment (aquatic life such as microbes) that may affect the degradation or migration of human DNA. A human epidermal tissue was used, and it experienced a natural decay process. The 15mL samples collected were deposited onto a Whatman® 0.2µm 47mm nylon membrane with vacuum filtration, and the paper was used for extraction, followed by a QIAGEN® DNeasy® Blood and Tissue kit. This kit is commonly used in environmental research laboratories, especially with environmental DNA (eDNA). Both the natural decaying process and the extraction were chosen to ensure a more realistic crime scene scenario. The concentrations of DNA at all intervals for each season were obtained in order to indicate any possible trends for further analysis. Only samples with higher DNA concentrations were processed for further analysis. Amplification was performed using the GlobalFiler® Polymerase Chain Reaction (PCR) amplification kit. Genotyping was performed using the SeqStudio™ genetic analyzer to indicate which Short Tandem Repeat (STR) loci show consistency between the known and unknown samples. This step is necessary to help ensure that the methods used are working and can be used to create a protocol for missing persons cases. This study aims to provide enough evidence that human DNA can be collected and extracted from an aqueous environment and still obtain a viable genotype for identification.

Preliminary tests showed that the highest concentration of DNA being secreted is coming from the skin of the sample and not the fat. This information concludes the skin area of the sample should be the same size for every trial, but the amount of fat present will not cause a large difference in the concentrations being observed. Results to date also indicate that the DNA concentration is higher at the interval that is closest to sample.

This research aims to implement new and more extensive searching protocols for detailed missing persons cases. The new protocol could increase the amount of missing persons cases solved yearly and employ the use of future eDNA cases toward a forensic outlook.

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### DNA, Degradation, Genotyping

## B129 The Implementation of Biological Models for the Probabilistic Interpretation of Next Generation Sequencing (NGS) Autosomal Short Tandem Repeat (aSTR) Mixtures

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**Learning Overview:** The goal of this presentation is to provide an overview of the models used in the probabilistic genotyping of NGS DNA profiles and show the results of mixture deconvolution using these models.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing continuing efforts in modeling of NGS profiles.

Forensic casework examination of biological samples often produces low-level, mixed, and complex DNA profiles. The process in which forensic scientists analyze the DNA evidence typically involves the amplification of Short Tandemly Repeated (STRs) lengths of DNA using a polymerase Chain Reaction (PCR) technique. Most commonly, the amplified products are separated by allele length using Capillary Electrophoresis (CE) techniques, and the resulting signals are visualized as an electropherogram (epg), showing peak heights for observed alleles.

Recently laboratories are adopting NGS, also known as Massive Parallel Sequencing (MPS), for DNA profiling. One of the advantages of applying NGS technology to forensic DNA typing is that the amplified STR products can be separated by the allele sequence rather than allele size. Sequenced-based information can increase discriminatory power of STRs. This discriminatory power has been demonstrated by showing that the Random Match Probability (RMP) was on average 700 times lower when considering sequence-based information, compared with using length-based allele designation,<sup>1</sup> and hence the single-source Likelihood Ratio (LR) was, on average, 700 times higher.<sup>1</sup> Furthermore, many more informative markers may be included in the PCR reactions including Y-chromosomal Short Tandem Repeats (Y-STRs), X-chromosomal Short Tandem Repeats (X-STRs), Single Nucleotide Polymorphisms (SNPs), and phenotypic markers to aid investigation of no-suspect or human-identification cases.<sup>2</sup>

Despite the appeal of sequencing technologies, there are few published models for the interpretation of autosomal STRs in NGS-DNA mixtures using probabilistic genotyping methods. At the time of writing, there is only one probabilistic genotyping software that has been modified to assist with the interpretation of NGS DNA profiles. Whereas there are a number of probabilistic genotyping software implementations used to assist with the interpretation of CE-DNA mixtures.<sup>3-11</sup>

While both CE and NGS methods use PCR techniques to amplify STRs, there are differences in the methodology that leads to differences in the characteristics of the observed profile such as stutter rates, DNA degradation, and locus specific amplification effects. There are a number of studies that have investigated these characteristics.<sup>12-19</sup> The studies help the community better understand how continuous models used in the interpretation of CE-DNA profiles may also be used for the interpretation of NGS DNA profiles.

This presentation describes the adaptation of the allele peak height model introduced by Bright et al. to develop an allele read count model for the continuous interpretation of autosomal STRs in NGS DNA profiles.<sup>20</sup> Bright et al.'s model consists of three key parameters: template, locus-specific amplification efficiencies, and degradation. In this adapted model for allele read counts, it considers how each of these parameters applies to NGS DNA profiles and shows the improved modeling of allele read counts using informed locus-specific amplification efficiency priors in a Markov chain Monte Carlo method.

This allele read count model is implemented in conjunction with the stutter modeling presented in Vilsen et al. and Cheng et al. into a probabilistic genotyping approach, with the goal of showing some results of the continuous interpretation of NGS DNA mixtures.<sup>13,18</sup>

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## NGS, Probabilistic Genotyping, Mixture Interpretation

## B130 An Alternate Workflow for Preparing Precision ID Identity and Ancestry Panel Libraries for Illumina® Sequencing

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**Learning Overview:** After attending this presentation, attendees will better understand about a verified workflow that can be used to generate Single Nucleotide Polymorphism (SNP) genotypes using Precision ID primer panels, third-party library construction reagents, and Illumina® sequencing instrumentation and reagents. This presentation will also highlight a published data analysis workflow that uses quality control measures and flagging to aid in genotype confirmation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how to incorporate this method as a cost-effective alternative to the complete commercial workflow, based on their laboratory's requirements and existing instrumentation. Given that the workflow presented is flexible, attendees could make adjustments in order to optimize scale and utilize accessible core sequencing lab instrumentation.

SNPs have been under development for human identification purposes for over a decade. Although they are not compatible with criminal casework databases such as the Combined DNA Index System (CODIS), they offer some advantages over the more traditionally typed Short Tandem Repeat (STR) markers: (1) they can be designed as very small Polymerase Chain Reaction (PCR) amplicons, offering greater success with degraded samples; (2) they do not generate stutter, an artifact of STR amplification that complicates data interpretation; and (3) they can offer predictions of biographic ancestry and phenotype, which could be valuable for generating investigative leads in criminal cases. The Precision ID NGS System, a commercially available workflow offered by Thermo Fisher Scientific™, offers a streamlined solution for genotyping forensically relevant identity and ancestry SNPs using next-generation sequencing. The Precision ID Identity and Ancestry Panels combined target 289 SNPs, and the sensitivity, reproducibility, and accuracy of the panels have been already evaluated by the forensics community. One potential limitation to the broad use of these panels is that genotyping using Thermo Fisher Scientific™ reagents and instrumentation requires a significant financial commitment. Thus, the aim of this study was to find an alternative workflow compatible with Illumina® sequencing chemistry for genotyping SNPs using the Precision ID Identity and Ancestry Panels, given the laboratory and nearly all core sequencing facilities have Illumina® instruments. Commercially available genomic DNA samples ( $n = 3$ ) were amplified with both panels in separate reactions using three commercially available uracil-tolerant polymerase master mixes. All resulting amplicons were prepared into libraries using the KAPA™ Hyper Prep Kit and sequenced via Illumina's® MiniSeq Sequencing System. Sequencing reads were analyzed using a previously published QIAGEN's® CLC Genomics Workbench workflow, and a Python® script was used to compile final genotypes and coverage information. Phusion™ U Multiplex PCR Master Mix statistically outperformed the other polymerase master mixes tested, with respect to the number of SNPs genotyped and associated coverage. To ensure that a workflow using the Phusion™ U Multiplex PCR Master Mix would be compatible across diverse sample types, this study optimized the number of PCR cycles using commercially available genomic DNAs, along with DNA from reference buccal swabs and environmental samples (total  $n = 12$ ). Optimized conditions yielded  $98.2 \pm 0.45\%$  autosomal SNP loci recovery in positive controls. The developed workflow should be straightforward to implement by forensic laboratories and suitable for processing reference and casework samples.

### Next Generation Sequencing, Single Nucleotide Polymorphisms, Alternate Workflow

## B131 “Touch Microbiome” vs. “Touch DNA”: Exploring Potentials and Limitations Toward Forensic Personal Identification

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**Learning Overview:** After attending this presentation, attendees will understand the potential and the limitations that microbiome studies have in providing information on the donor who touched an item (“touch microbiome”) in comparison with the standard DNA fingerprinting methods (“touch DNA”).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of competence by showcasing the importance of the study and analysis of the “touch microbiome” as an alternative/integrative way to obtain intelligence information useful for solving forensic caseworks, overcoming the limitations involving “touch DNA” evidence.

The rising usage of Next Generation Sequencing (NGS) in forensic contexts allowed for the analysis of microorganisms to become an auxiliary tool to conduct personal identification analyses.<sup>1</sup> Among the microbiota easy to find at the crime scene, the skin “microbial footprint” is highly individual, stable over time, and easily released into the surrounding environment. Microbiota analyses have been successfully admitted as evidence in court cases, but the lack of accurate forensic reference databases currently limits their use.<sup>2,3</sup> On the other side, the increased sensitivity in the simultaneous amplification of different Short Tandem Repeats (STR) has enabled forensic geneticists to recover DNA profiles from highly degraded samples and low DNA content evidence, including those generated when a person touches a surface or an object (“touch DNA”). Although the forensic community has been talking about “touch DNA” for over 20 years, there are still some dark sides, such as the origin of this DNA, its transfer dynamics, its ability to withstand different environmental conditions, and the risks associated with its secondary transfer.<sup>4,5</sup>

This study wanted to compare the sensitivity and specificity of these two different methods (“touch microbiome” versus “touch DNA”) for forensic identification purposes and highlight their strength and weaknesses. Eleven volunteers of both sexes and of different ages filled in a questionnaire on their lifestyles and on any previous or current pathologies and treatments they had. Each individual’s palm and fingers were sampled using three sterile swabs (“skin sample”), then volunteers deposited their fingerprints on a glass slide that was then swabbed (“fingerprint sample”). Microbiome DNA and human DNA were extracted respectively using QIAamp® PowerFecal Pro DNA and ChargeSwitch® Forensic DNA from each sample. DNA was quantified with NanoDrop™, then the V4 region of 16S rRNA gene was analyzed using the Illumina® MiSeq® platform whereas human STRs were amplified with the AmpFℓSTR® NGM SElect™. NGS data were analyzed using R scripts normally used in metabarcoding ecology studies.

This study obtained good quality microbiome profiles from 20 out of 22 samples analyzed and found a significant difference between the composition of the “skin” and the “fingerprint” samples. Timing between washing and samplings showed an effect on the relative abundance of *Actinobacteria spp.* in both sample types, and pairwise comparisons showed a relative increase of *Proteobacteria spp.* and decrease of *Firmicutes spp.* and *Actinobacteria spp.* in fingerprint samples versus their skin counterpart. STR profiling of “touch DNA” samples showed discrepancies between skin and fingerprint samples (e.g., missing peaks for some markers in all samples but one).

While additional analyses of the results are currently in progress, this study can anticipate that microbiome profiles provided information that could potentially be used to identify an individual where “touch DNA” also failed.

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### Microbiome, Touch DNA, Next Generation Sequencing

## B132 Massively Parallel Sequencing (MPS) of Diatom and Bacterial DNA: A New Approach for Freshwater Trace Evidence Analysis

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the value of bacterial and diatom DNA metabarcoding and MPS for the comparison and exclusion of freshwater trace evidence with questioned environments

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the rapid and discriminatory potential of molecular approaches for crime reconstruction in freshwater environments. This presentation will outline a novel MPS protocol, targeting *rbcL* and 16S rRNA markers, applied to bulk environmental and trace forensic samples to indicate the site of initial transfer.

Freshwater environments are frequently encountered in forensic cases as the location of death or an evidence deposition site. Although they are diverse and widespread, few forensic techniques are readily available to link persons, items, and scenes of forensic interest. Environmental bacterial and diatom communities transferred to a questioned sample may yield a powerful discriminatory tool.<sup>1</sup> Although MPS of bacterial communities has shown great promise in time of death estimations and in soil trace evidence comparisons, the potential for bacterial markers to infer a site of freshwater evidence transfer has not yet been explored.<sup>2,3</sup> Diatoms, an abundant and species-rich group of microscopic algae, are more frequently studied as a form of aquatic trace evidence and are useful indicators of drowning in forensic pathology.<sup>4</sup> While analysis typically uses microscopy to assess diatom morphology and surface structures, this approach is time consuming with few forensic experts available to support accurate species identification. To overcome these limitations, and to increase the potential for more diverse forensic applications, it is imperative to test and develop innovative technologies such as MPS for the analysis of freshwater evidence.

This research represents the first characterization of diatom DNA in forensic samples and investigates the reliability of diatom and bacterial sequencing to discriminate between freshwater environments and indicate the transfer site of immersed clothing items. An MPS protocol was developed for application to bulk environmental and trace forensic samples. Eighteen freshwater sites in and around Adelaide (South, Australia) were sampled in June 2019 and a 5L disturbed water sample collected from each. Four 100% cotton t-shirts were immersed in four of these environments and retained for analysis. DNA was extracted from a concentrated water sample and two different treatment methods were trialed to recover DNA from each clothing item. 16S rRNA (bacteria) and *rbcL* (diatom) gene regions were amplified and sequenced using Illumina® MiSeq®. Species were identified using the available reference databases and the relative abundance of each determined. To support reliable interpretations, statistical approaches including source-tracking, and likelihood ratio calculations were incorporated.

The results demonstrate the successful recovery and sequencing of both diatom and bacterial DNA from all environmental and forensic samples. The 18 freshwater sites were discriminated based on the overall species assemblage, and each clothing sample was successfully predicted to the correct control site regardless of the DNA recovery method used. Diatom DNA proved a more stable molecular marker on the questioned samples compared to bacterial DNA, although the inclusion of a second taxonomic marker offered an independent tool to enhance sample comparisons and exclusions. This presentation represents the first application of MPS and the first multi-organismal study of freshwater trace evidence samples for crime reconstructions. The technique offers a rapid and less subjective tool for forensic analyses of environmental markers, with future avenues for research highlighted.

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### Massively Parallel Sequencing, Forensic Ecology, Trace Evidence



## **B133 The Dive Into Next Generation Sequencing (NGS): From Validation to Implementation**

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**Learning Overview:** After attending this presentation, attendees will understand how NGS works and the many different applications for which it can be used. Attendees will also gain insight into the validation and implementation process using this technology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a demonstration of NGS's ability to augment traditional DNA technology, such as Short Tandem Repeats (STRs). Furthermore, utilizing this technology may assist in providing investigative leads to law enforcement through the use of additional platforms, including genealogy, whole genome mitochondrial DNA (mtDNA) sequencing, phenotypic markers, and ancestry markers.

For almost a quarter of a century, DNA analysis has been dominated by STR and Single Nucleotide Polymorphism (SNP) testing, but it is time to dive into a new era to run in tandem with traditional DNA technology. Utilizing NGS, hundreds of forensic loci are targeted and amplified, including the core locations that have been employed in DNA testing for years. This is important as it allows for compatibility between the current DNA database formats and NGS technology. In addition, NGS can be applied in multiple platforms, which provides the ability to target other sections of DNA, including ancestry markers, phenotypic markers, and whole genome mitochondrial DNA. This technology may heat up cold cases by providing new leads, especially in unidentified remains or unsolved cold cases where a comparable mtDNA profile may establish maternal lineage or genealogy can determine a potential family member leading to identification. With these eye-opening benefits, three different platforms of NGS are being validated and implemented including the Verogen® ForenSeq™ DNA Signature Prep Kit, mtDNA Whole Genome Kit, and a Forensic Genetic Genealogy (FGG) system. The validation studies were designed in accordance with the recommendation of the Scientific Working Group on DNA Analysis Methods (SWGDM) and the Federal Bureau of Investigation (FBI) Quality Assurance Standards (FBI QAS), including a study that serves to evaluate the effect of processing less samples on a single run in order to obtain more reads per sample. A disadvantage to NGS as opposed to traditional STR testing is the amount of hands-on time that is required by the scientist. To provide a high throughput option for NGS processing, the Aurora Biomed® Versa™ 1000 robotic platform was also evaluated as an alternative to a manual setup. This presentation will review the validation process used to bring online three different NGS platforms as well as the implementation process in the laboratory.

### **Next Generation Sequencing, Mitochondrial DNA, Genealogy**

## B134 An Evaluation of Hotspot Chloroplast Regions to Differentiate Crop Type and Biogeographical Origin of *Cannabis Sativa*

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**Learning Overview:** After attending this presentation, attendees will understand the basic principles behind utilizing DNA barcoding and Massively Parallel Sequencing (MPS) techniques to develop genotyping methods to aid in the differentiation of crop type and biogeographical origin of *Cannabis sativa*.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the applicability of chloroplast markers and an MPS panel that could potentially assist law enforcement agencies in distinguishing legal hemp and illicit marijuana and also aid in the linkage of illegal cases and drug trafficking.

*Cannabis sativa* (marijuana and hemp) is one of the most controversial crops worldwide. In the United States, the state-specific legalization of marijuana and recently legalized hemp poses a problem for law enforcement. The study also contributed to the development of a *C. sativa* chloroplast DNA (cpDNA) database, which will need to be expanded to include more chloroplast markers as well as genotypes from samples obtained around the world.

DNA barcoding is a technique that examines specific regions of DNA to identify inter- and intra-species genetic differences. Both cpDNA and mitochondrial DNA have been studied for species identification, but cpDNA is particularly beneficial for plant barcoding markers. However, due to the limited discriminatory ability of common barcoding markers in plant identification, this study sought to discover more informative polymorphic regions that could be used for cannabis differentiation. By comparing published whole genome cpDNA sequences, seven polymorphic hotspot regions were identified. Capillary Electrophoresis (CE)-based methods were developed to quickly genotype the polymorphic loci from the hotspot regions in hemp samples from the United States and Canada, marijuana samples from Mexico and Chile, and medical marijuana samples from Chile. The effectiveness of the assays to distinguish sample groups was assessed using haplotype analysis, pairwise comparisons, and principal component analysis. While CE offers a reliable and robust technique, it has disadvantages such as limited multiplexing capability and size-based genotyping. MPS is an emerging technology in the field of forensic genetics that processes distinct advantages such as high-throughput multiplex capacity and the potential to provide deeper interrogation of sequence-based polymorphisms. Therefore, this study also reports on the development of a MiSeq® FGx® assay targeting seven hotspot regions in the *C. sativa* chloroplast genome.

This study developed six fragment analysis assays and four SNaPshot™ assays for 25 polymorphisms in the seven hotspot regions. The genotype results of 166 samples from five different regions indicated a clear separation of Canadian hemp from other groups. Results also demonstrated that increasing the number of markers was able to improve the discriminatory power of the assays. Moreover, this study reports preliminary results from sequencing 14 hemp and marijuana samples from different countries using a novel MPS assay. A total of 49 polymorphisms were observed in the seven hotspot regions, 16 of which have not been previously reported using CE. Additionally, isoalleles, which were able to differentiate two samples that had the same haplotype using CE-based methods, were discovered. However, no combination of markers was capable of definitively distinguishing the crop type and biogeographical origin of *C. sativa*, and a more extensive database is needed to evaluate the true discriminatory power of these markers.

Overall, this research investigates the seven hotspot regions in the *C. sativa* chloroplast genome from five different origins and developed DNA barcoding and MPS assays that can potentially be used for exclusionary purposes in marijuana identification and geosourcing.

### *Cannabis Sativa*, DNA Barcoding, Massively Parallel Sequencing



## **B135 The Recovery of Full Single Source DNA Profiles From Contributors to Complex Mixtures by Direct Single Cell Subsampling (DSCS)**

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**Learning Overview:** After attending this presentation, attendees will better understand a novel DNA mixture deconvolution tool that relies on subsampling of individual cells by physical capture, subsequent high-sensitivity DNA typing, and quantitative computer interpretation to extricate fully probative single source genotypes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees how the DSCS approach could permit forensic scientists to reduce genotype information loss in standard mixture analysis caused by excessive numbers of overlapping alleles and/or the presence of low-level minor contributors, with some of the latter not even being detectable by standard mixture analysis.

DNA mixtures are often difficult to deconvolute and interpret due to the presence of overlapping alleles from multiple contributors and stutter artifacts. Therefore, standard mixture interpretation now requires the use of Probabilistic Genotyping (PG) software. However, even with the use of PG software, genetic information loss is often seen in some complex mixtures by decreased likelihood ratios in comparison to single source genotype profiles. In order to aid in mixture deconvolution and genotype information loss in complex mixtures, a simple micromanipulation technique referred to as DSCS has been developed. This technique allows for manual separation of individual cells or subsets of cells (typically 2–3) from mixtures prior to DNA typing paired with a subsequent Low Copy Number (LCN) technique in order to attempt to obtain single source DNA genotype profiles from all contributors to a mixture.

The feasibility of this approach will be presented by first using a simple two-person 1:1 buccal cell mixtures that were not fully resolvable by PG. Single and multiple cells were recovered and analyzed via DSCS and full single source profiles were achieved for each donor. The DSCS approach was then applied to more complex equi-proportional 3–6 person mixtures (i.e., 1:1:1, 1:1:1:1, 1:1:1:1:1, and 1:1:1:1:1:1). PG software, STRmix™, and EuroForMix were utilized to compare the information loss (in bans) of the standard “bulk” mixtures to that of the single source reference profiles. Comparison to the analyzed DSCS samples obtained indicated instances in which the DSCS method resulted in a substantial gain of probative information. The gain of information was maximized in some instances by co-inferring genotypes utilizing a joint likelihood function. The mixture and DSCS analysis conditions under which this occurred will be described. In addition to obtaining single source profiles, the DSCS sampling method can also result in subsample “mini mixture” byproducts. PG methods can then be utilized to analyze these mini mixtures. For example, a three-cell subsample could, depending upon the number of donors, result in cells originating from a single donor, two donors, or three donors. These artificial mini mixture subsamples may be significantly reduced in complexity compared to the original mixture by reducing the number of contributors present or creating more unbalanced weight ratios (e.g., in a three-person mixture, some of the three-cell mini mixtures recovered comprise only two of the three donors at weight ratios of 2:1 rather than 1:1). The PG results from these mini mixtures can be used to provide additional support to the inferences made from the single source DSCS results. The mini mixture analyses for each of the complex mixtures studied will be presented.

The DSCS approach could permit forensic scientists to reduce genotype information loss in standard mixture analysis caused by excessive numbers of overlapping alleles and/or the presence of low-level minor contributors, with some of the latter not even being detectable by standard mixture analysis.

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**Mixtures, Micromanipulation, Single Cell**

## **B136 Evaluating the Forensim Package for Modeling DNA Mixtures for Statistical Calculations of Contributor Attribution in North American Populations**

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**Learning Overview:** After attending this presentation, attendees will understand the functionality and efficacy of the forensim R package for application to forensically relevant DNA mixture simulations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an external evaluation of the functionality of the forensim R package to generate DNA mixture profiles and genotypes based on population-based Short Tandem Repeat (STR) allele dynamics. As laboratories improve DNA mixture deconvolution efforts, there is a critical need to evaluate kit deconvolution capabilities using modeling programs and algorithms in addition to benchtop artificial mixtures. These approaches leverage population dynamics and ensure that this study's conclusions are accurate and reproducible in casework settings.

Statistical modeling and simulations are common tools employed in forensic genetics to assess the validity and accuracy of DNA mixture deconvolution approaches. There is growing interest in understanding how individual genotypes and alleles from different population groups can be over or underrepresented in DNA mixture profiles. Using mixed ancestry mixtures, it has been demonstrated that populations with lower genetic diversity are overrepresented in mixture deconvolution match statistics. There remains some uncertainty however, as to how minor contributors match to low diversity populations in models assuming admixed major and minor contributors. Furthermore, systematic ancestry proportions remain underinvestigated; this study hypothesizes that systematically increasing the African versus European ancestry proportion of minor contributors (i.e., 5%–50% in 5% increments) will reveal biases in minor contributor match statistics influenced by African ancestry proportion. As complex DNA mixtures are more routinely interpreted, it is important to understand how statistical modeling and simulation(s) represent genotype frequency distributions in the ancestral affiliation of each mixture contributor.

In this study, a detailed evaluation was conducted to determine whether the forensim R package is capable of modeling complex DNA mixtures from known population data. In R, forensim provides a series of simulation tools to generate genetic mixtures such as those encountered in forensic casework. Genotypes are derived from population allele frequencies and population structure. Further, the package also allows for the interpretation of complex Short Tandem Repeat (STR) results by providing a maximum likelihood estimator for the number of contributors to a mixture, random man non-excluded probabilities, and likelihood ratios for low template samples with drop-out, drop-in, and varying number of contributors and replicates. Recent studies using forensim demonstrate that underrepresented population(s) (i.e., those populations with lower genetic diversity) may be overrepresented in DNA mixture match statistics due to limited allele diversity in their population datasets. Of particular interest, DNA mixture likelihood ratios were demonstrated to suggest that non-contributors from lower genetic diversity populations are included in the mixture(s), so a detailed investigation into the possibility of false positive(s) and negative(s) in a series of simulated DNA mixtures from five populations has been performed. Using known genotypes and allele frequencies from previously published population datasets, this study compared genotypes, heterozygosities, and allele combinations to evaluate model accuracy given variable input population information. Likelihood ratios were calculated to assess whether non-contributors were included within the DNA mixture profiles as a measure of quantifying false positive rates across population groups and number of contributors.

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### **DNA Mixtures, Mixture Simulations, Allele Frequencies**

## B137 The False Inclusion of Non-Contributors in DNA Mixtures Cases

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**Learning Overview:** The goal of this presentation is to assess the risk of false inclusions when comparing DNA profiles of unrelated persons to simulated mixed traces in Lebanon.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) shedding light on the effect of allele sharing and homozygosity on the evaluation of DNA mixtures, especially in consanguineous and endogamous communities; (2) shedding light on the risk of false inclusions in DNA mixtures in similar populations; and (3) providing recommendations in similar populations concerning the use of profile-to-mixture matching studies.

The interpretation of forensic DNA mixtures is particularly complex due to several factors, including allele sharing, allele dropout, low copy number, and Polymerase Chain Reaction (PCR) stutter artifacts. Statistical interpretation is essential in reporting DNA results in which various approaches are used to assess the weight of the resulting mixed DNA profile. The present research aimed at assessing the risk of false inclusions when comparing DNA profiles of unrelated persons to simulated mixed traces in Lebanon.

A total of 517 samples were collected from seven Lebanese villages representing different Lebanese regions and religious backgrounds. Profiling was performed with three different profile sizes: 16, 23, and 28 Short Tandem Repeat (STR) systems using three multiplex STR kits: the PowerPlex®16 HS, the PowerPlex® ESI 17, and the PowerPlex® CS7. A specialized in-house software, the DNA Mixture and Matching Simulator, was developed to perform simulation studies, where each individual profile was compared against electronically simulated mixtures of two contributors.

The comparisons of the individual profiles to mixtures of two contributors showed false inclusions with 16-, 24-, and even 29-locus profiles. Sixty-nine non-contributors were falsely included in DNA mixtures with 16 tested loci. With 24 tested loci, 13 non-contributors were falsely included in the mixtures. Five non-contributors were falsely included in DNA mixtures using 29 STR systems. This showed that increasing the number of STR loci increases the power of discrimination and reduces the possibility of false inclusions, but it does not eliminate it.

Physical DNA mixtures of the electronically simulated cases were produced and statistical interpretation was then performed based on the semi-continuous model using three of the most frequently used DNA mixture analysis expert software programs, taking into account the relevant allele Lebanese frequency and inbreeding coefficient of the population, to compute the likelihood ratio. The statistical results of the expert software confirmed the inclusion of non-contributors in the cases with adventitious matches and confirmed these false inclusions.

DNA mixture simulations of three contributors were then performed using the same in-house software where each individual profile was compared against electronically simulated mixtures of three contributors. The comparisons showed 1,100 cases in which a non-contributor was falsely included in a DNA mixture of three contributors using 29 STR systems. Moreover, a challenging case showed eight non-contributors falsely included using 24-locus profiles and five non-contributors falsely included using 29-locus profiles.

These false inclusion cases shed light on the effect of allele sharing and homozygosity on the evaluation of DNA mixtures, especially in consanguineous and endogamous communities. In similar populations, this study recommended using profile-to-mixture matching studies for exclusion purposes only, even when using 29-locus profiles and expert software statistical analysis.

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### Forensic DNA, Mixed Traces, Expert Software

## B138 DNA Mixture Interpretation: Bias Versus the Scientific Method

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**Learning Overview:** The goal of this presentation is to highlight the detrimental effect on justice when forensic experts do not follow the scientific method during analysis and interpretation of results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware that it is only via the use of the scientific method that forensic science can serve justice.

The challenges related to the interpretation of DNA mixtures are real and multiple. However, there are cases in which the DNA expert can interpret a DNA mixture with relative ease and convey the information to the court in as unbiased a manner as possible. It is the onus of the DNA expert to present the court with all the scientific evidence. When only one side of the evidence is presented in court, thus contrary to the scientific method, the evidence presented by the DNA expert should be placed into question. This is evident of cognitive bias and flawed interpretation standards implemented by that institution.

In 2017, a case was heard in the Cape Town High Court of South Africa, where three members (both parents and one son) of a family were murdered in 2015. The second son in the family was the accused. DNA evidence was processed (Profiler Plus® kit) with only nine Short Tandem Repeat (STR) markers. In this family, there were only five informative STR loci between the mother and her sons. This called for careful examination of all available data when interpreting these three DNA profiles, as the scientific method dictates.

During the case of the State, Expert A testified that the DNA profiles of the mother and two sons: “... can be read into this mixture.” This mixture was on a swab from the shower of the bedroom shared by the sons. This opened the door to speculation by the prosecution that the accused showered after allegedly committing the murders, thus depositing his mother’s DNA in the shower. Expert A testified that she used a Sample Status Report (SSR) containing information related to this DNA mixture to write her affidavit.

However, it is evident from a qualitative comparison of the DNA profiles that the mixture could have resulted from the DNA profiles of the two sons alone. It is by chance that the two brothers inherited different alleles from their mother at each of the five informative loci. During cross-examination, Expert A conceded that an alternative interpretation could have been that the DNA mixture consisted only of the DNA profiles of the two brothers. She testified that it is for “... the quality office to decide” whether alternative interpretations should also be mentioned in her affidavit.

The SSR used by the DNA Expert A contained qualitative data, but also quantitative information based on quantitative Polymerase Chain Reaction (qPCR) analysis (Quantifiler® Duo DNA Quantification kit). When calculating the male-to-female DNA ratio, it is evident from the data that no female DNA was present in the DNA mixture.

Independent Expert B, when analyzing the mixture and reference profiles, took both the qualitative and quantitative data into consideration and concluded that only the DNA profiles of the two sons were present in the mixture.

Intentionally or not, Expert A omitted to use the quantitative data in her interpretation. This case was not the first time that Expert A omitted using all the data. In 2013, the judge stated in his judgment that “This quantitative [sic] element of the interpretation of the electropherograms was not taken into account ...,” referring to Expert A, ultimately favoring the evidence of another expert who took all data into account.<sup>1</sup>

The interpretation of DNA mixtures is often a challenge and susceptible to cognitive bias. However, in this case, the evidence was clear but the scientific method was ignored. If an expert continues to ignore part of the evidence, after this bias has been highlighted in a previous case, it is possible that the danger of cognitive bias is not fully comprehended or is deliberately embraced to support a specific view in court. Whatever the case may have been, it is evident that quality standards from the expert’s institution do not address the management of cognitive bias, nor are they in line with the scientific method.

It is only by embracing the scientific method and managing cognitive bias that DNA experts will enable the pursuit of justice through truth in DNA evidence.

### Reference(s):

<sup>1</sup> *Bokolo v State* (483/12) [2013] ZASCA 115 (18 September 2013).

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### DNA Mixtures, Interpretation, Bias

## B139 Testing, Comparing, and Validating Traditional and Emerging Number Of Contributors (NOC) Systems for Forensic DNA Purposes

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**Learning Overview:** After attending this presentation, attendees will better understand the *A Posteriori* Probability distribution on  $n$  ( $APP(n)$ ) and become aware of methods by which to validate and benchmark NOC systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by summarizing the results acquired from a developmental validation of a method that estimates the  $APP(n)$  and by demonstrating that it significantly outperforms the allele counting method and an Artificial Neural Network (ANN).

Forensic DNA signal is notoriously challenging to interpret and requires the implementation of computational tools that support its interpretation. While data from high-copy, low-contributor samples result in electropherogram signal that imparts informative and unambiguous weights of evidence, electropherogram signal from forensic stains are often garnered from low-copy, high-contributor-number samples that are often obfuscated by allele sharing, allele drop-out, stutter, and noise peaks. Since forensic DNA profiles are often too complicated to quantitatively assess by manual methods, continuous, probabilistic frameworks that draw inferences on the NOC and compute the Likelihood Ratio (LR) given the prosecution's and defense's hypotheses have been developed.

This presentation summarizes the validation results acquired from the NOCIt inference platform, which determines an *a posteriori* probability distribution on the number of contributors given an electropherogram ( $APP(n)$ ). NOCIt is a continuous inference system that incorporates models of peak height (including degradation and differential degradation), forward and reverse stutter, noise, and allelic drop-out while considering allele frequencies in a reference population. The system's performance was assessed by evaluating the  $APP(n)$ 's unimodality, sensitivity, fall-out (i.e., number of incorrect  $n$  included in downstream LR determinations), repeatability, and runtime for 815 publicly available PROVEDIt samples (lftdi.com), consisting of degraded, Ultraviolet (UV)-damaged, inhibited, differentially degraded, or uncompromised DNA mixture samples containing up to five contributors. This probabilistic system makes repeatable and reliable inferences about the NOCs and significantly outperforms traditional counting methods that render minNOC. For example, the proportion of samples for which the maximum  $APP(n)$  was at  $n = \text{TrueNOC}$  was 0.80, while the proportion of samples for which  $\text{minNOC} = \text{TrueNOC}$  was 0.56. The  $APP(n)$  was also compared to a fast and repeatable emerging technique—an ANN—which resulted in a wide distribution on  $n$  making the system highly sensitive but with high fall-out.

Lastly, unlike counting or ANNs, the  $APP(n)$  can be used in conjunction with a downstream Likelihood Ratio (LR) inference system that employs the same probabilistic model, allowing for a full evaluation across multiple contributor numbers. This presentation will, therefore, illustrate the power of modern probabilistic systems to report a reasonable and contiguous range of  $n$  that explains the evidence, while introducing the automated features designed to ease burdens associated with artifact filtering.

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### Forensic DNA, Number of Contributors, Validation

## **B140 Evaluating Likelihood Ratios, Decision Thresholds, and Fire Debris Analysts’ Performances**

*Mary R. Williams, MS\*, National Center for Forensic Science, Orlando, FL 32816-2367; Michael E. Sigman, PhD, University of Central Florida, Orlando, FL 32816; Frances Whitehead, BS, University of Central Florida, Orlando, FL 32816-2367*

**Learning Overview:** After attending this presentation, attendees will understand how to evaluate the diagnostic performance of fire debris analysts with the aid of a ground truth database and a score-based decision threshold.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by graphically demonstrating the relationship between evidential value, weight of the evidence, and a score-based decision threshold. An understanding of these relationships is important for practicing forensic analysts that are required to develop and perform robust diagnostic analysis. Attendees will understand how to implement a simple method for validating an analyst’s performance, setting decision thresholds, and determining the associated error rates.

In recent years, forensic science research has often placed an emphasis on determining evidential value and limiting reporting to probabilistic statements of evidential values rather than diagnostic statements that represent a decision on the part of the analyst. Yet, many forensic science disciplines and the courts in the United States require analysts to continue making diagnostic statements about evidence (i.e., a “sample is positive for ignitable liquid residue” as opposed to “the probability of observing the evidence is 100 times greater in samples containing ignitable liquid residue than in samples that contain no ignitable liquid residue.”). The relationship between evidential value, strength of evidence, and the decision threshold for diagnostic statements is easily understood through the construct of decision theory based on Receiver Operating Characteristic (ROC) curves.

The ROC curve is a plot of the true positive rate as a function of the false positive rate for a set of diagnostic decisions made at a corresponding set of decision thresholds. The ROC curve describes the performance of a binary (two state) system, which lends itself to the legal framework where questions are often posed in binary form. Establishing a ROC curve requires two factors: (1) a set of ground truth samples belonging to the two states, and (2) a scoring system that reflects the weight of the evidence relative to the two states. In some cases, ground truth forensic samples may be difficult to obtain; however, a new database of ground truth fire debris samples exists and will be introduced in this presentation. A seven-point discrete confidence rating scale for sample scoring will be applied to ground truth fire debris samples. Sample analysis will implement the American Society for Testing and Materials (ASTM) E1618 Standard Method coupled with principles of linear sequential unmasking. ROC curves, optimal decision thresholds, and error rates will be presented for three test analysts with differing experience levels. The general methodology is well established in published literature and applicable across many forensic disciplines.

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### **Fire Debris Analysis, Performance Validation, Decision Theory**

## B141 Validating Fire Debris Classification With Ground Truth Samples

Michael E. Sigman, PhD\*, University of Central Florida, Orlando, FL 32816; Mary R. Williams, MS, National Center for Forensic Science, Orlando, FL 32816-2367

**Learning Overview:** After attending this presentation, attendees will understand how to validate fire debris classification using machine learning methods and ground truth samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new publicly available fire debris database and demonstrating the use of the database records to validate fire debris classification as positive or negative for the presence of ignitable liquid residue by machine learning methods.

Validating the performance of computational classification methods requires the use of casework-relevant samples with known ground truth class assignment for each sample. In the case of fire debris analysis, the samples must each contain fire debris from burned substrate materials and possibly an ignitable liquid residue, the presence or absence of ignitable liquid residue must be known, the relative strength and the degree of weathering of any ignitable liquid contribution must be representative of casework. A database of laboratory-created fire debris records that meet these criteria has recently been released for free public access online by the National Center for Forensic Science. Uses of the new database for training and validation will be highlighted in brief, and use of the database records for validation of machine learning classification methods will be explored in more detail.

Machine learning methods will be described for classifying database samples as positive or negative for ignitable liquid residue based on decision theory and estimates of the sample evidentiary value. Multiple bootstrapped training sets were created from the fire debris database records for each computational method. The multiple data sets were each split 80:20 for training and cross validation of a machine learning method. Following training and cross validation, the models were then used to evaluate a set of 129 fire debris samples from experimental large-scale burns. The mean and standard deviation for the calculated log likelihood ratios for the large-scale burn samples were then compared with the interpretation of an informed analyst. Machine learning methods investigated in this work include k-nearest neighbors, linear and quadratic discriminant analysis, and support vector machines. The average areas under the receiver operating characteristic curves for each machine learning method ranged from 0.78 to 0.92 with relative standard deviations of less than 6.5%.

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### Fire Debris Analysis, Performance Validation, Machine Learning

## **B142 Absorbent Household Materials for the Collection of Ignitable Liquid Residues (ILRs) From Surfaces of Varying Porosity**

*Jessica Carlotti, BS\*, Rineyville, KY 40162; Cynthia J. Kaeser Tran, PhD, Eastern Kentucky University, Richmond, KY 40475*

**Learning Overview:** After attending this presentation, attendees will better understand the collection practices of ILR evidence from an arson scene, the possible use of household items as lifting materials to recover Ignitable Liquids (ILs) from surfaces of varying porosity, and the ability to simplify and improve the collection and recovery process of a suspected IL.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new practice through the use of common household absorbent materials in the collection of an IL present at an arson scene.

ILs are petroleum-based hydrocarbon mixtures such as gasoline, diesel, kerosene, paint thinners, and other fuel sources. These are used as accelerants to burn items at a quickened rate. The presence of ILs at the scene of a fire may indicate to investigators that arson has occurred. The residues from these ILs may remain on surfaces after the fire has been extinguished. Of particular interest are semi-porous surfaces such as concrete or wood that contain small pockets of pores which entrap unburned residues of ILs. The substrate containing the IL is collected from the scene and placed into a tightly sealed container for transport. Analysts identify the composition of the ILR through instrumentation such as Gas Chromatography/Mass Spectrometry (GC/MS).

Currently, surfaces suspected to contain ILR are taken back to the lab after removing a small portion from the scene of a fire. This method takes time, man power, often involves heavy machinery and tools, and can lead to contamination or destruction of the evidence. Being able to collect an ILR directly from a surface using an absorbent material will allow for easier transportation of evidence. Ideally, the absorbent material should be low cost, reproducibly manufactured, easy to use, and provide high collection and recovery efficiencies. The material utilized for this removal should not alter the chemical composition of the IL or contain any interferences once the IL is collected from a surface. These criteria will allow for a chemical classification to be made based on the hydrocarbon composition of the recovered IL.

Determining an appropriate material to use for the lifting of ILs from a surface requires several aspects to be optimized, including the texture of each surface, diameter and depth of any pores present, and the size and texture of each lifting material. The IL that resides within pores will likely not be reached by large materials that penetrate the pores themselves. Smaller materials, especially granular ones, may not easily be removed from the pores once they make contact with the IL. Materials consisting of textured surfaces such as cotton matting and polypropylene cloths have exhibited better success for the collection of ILs.

This research examines readily available household absorbent materials such as microfiber and paper-based products that can be used to effectively collect ILRs. The microfiber and paper fibers of the absorbent materials penetrate the pores of the substrate allowing contact with the ILR. The attachment of fibers to a solid backing ensures the fibers will be easily removable from the pores, which may lead to an improved collection efficiency of an IL from a semi-porous surface. Overall, 12 materials of various chemical compositions were selected and taken through a series of analyses to determine the absorption and collection capabilities. While cellulose-based and synthetic fiber materials are most promising, this presentation will outline the results of a series of validations for each material and recommend which materials provide sufficient recovery of an IL from common surface types (non-porous, semi-porous, and/or porous).

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### **Ignitable Liquid Residue, Arson Collection Materials, Absorbent Materials**



## **B143 The Development of an Internet-Deployed Statistical Application for the Analysis of Compounds in Fire Debris Samples**

*Michelle Corbally, MS\*, National Center for Forensic Science, Orlando, FL 32828; Jessica Chappell, PhD, Alexandria, VA 22303; Mary R. Williams, MS, National Center for Forensic Science, Orlando, FL 32816-2367; Michael E. Sigman, PhD, University of Central Florida, Orlando, FL 32816*

**Learning Overview:** After attending this presentation, attendees will understand how to interpret data using an internet-deployed statistical application developed to analyze fire debris samples for compounds found in substrates and ignitable liquids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of using user-friendly statistical applications for the analysis of fire debris samples. This application provides a streamlined method for determining the presence, in fire debris, of up to 295 commonly identified compounds found in ignitable liquids and substrates. Compounds were identified by Target Factor Analysis (TFA) and required to meet or exceed a decision threshold based on a Fisher z-score identified by decision theory.

The application first converts the retention times of chromatograms into retention indices using a hydrocarbon ladder to allow for interlaboratory sample analysis. Peaks were detected in the Total Ion Chromatogram (TIC) and Extracted Ion Profiles (EIPs) based on second derivative maxima with intensities that exceeded  $\pm 2.5$  standard deviations from the mean. For a compound to be identified as corresponding to a chromatographic peak, two criteria are required to be met. The first criterion is that the compound being identified is within a  $\pm 11.157$  retention index range of the library compound that is being identified within the chromatogram. The second requirement is that the Fisher transform of the Pearson product moment correlation coefficient between the library Mass Spectral (MS) target factor and the vector resulting from projecting the target factor into the PC space meets or exceeds the optimized Fisher z-score threshold. The acceptable retention index range was based on commonly observed peak widths in the Ignitable Liquids Reference Collection (ILRC) database and Substrate databases. The optimal Fisher score was determined from receiver operating characteristic analysis of a set of standard-verified compounds in ignitable liquid and substrate samples to determine the optimal decision threshold.

The National Center for Forensic Science (NCFS) ILRC and Substrate database records were also analyzed using the statistical method implemented in the application to determine the frequency at which the 295 compounds were found in ignitable liquids and substrates. Approximately 1,050 ignitable liquids at different stages of weathering and 553 substrates burned using a Modified Destructive Distillation Method (MDDM) were analyzed. The retention times were converted to retention index using the slope and y-intercept of the retention times of the n-alkanes in American Society for Testing and Materials (ASTM) E1618 Test Mix Standard Reference Material (SRM) 2285.

The tool is deployable on the internet as a Shiny application and is synergistic with current fire debris analysis methods based on ASTM E1618. The target factor analysis used in the application developed from this research aspires to contribute an objective means for fire debris analysts to identify ignitable liquid and substrate compounds in fire debris samples.

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### **Fire Debris, Target Factor Analysis, Chemometrics**

## B144 A Chemical Waste Treatment Facility Fire and Explosion

*Chin Chin Lim\**, *The Forensic Experts Group, Singapore 577177, SINGAPORE*; *Michael Ming Kiong Tay, The Forensic Experts Group, Singapore 577177, SINGAPORE*; *Hui Ying Yvonne Sim, The Forensic Experts Group, Singapore 577177, SINGAPORE*

**Learning Overview:** After attending this presentation, attendees will be aware that although little information may be available for a case, identifying the key aspects of the case, exploratory analysis, and designing appropriate simulation experiments can turn a case around. This bottom-up approach gathers details and data, extracts information, and applies domain knowledge to obtain answers and insights to questions. Attendees will realize that a single event seldom leads to an incident. The latter is usually triggered by an event, followed by a series or cascade of related confluent events and conditions (i.e., a domino effect).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing the processes behind determining the contributory factors and root cause of the fire and explosion.

Fires and explosions occurring within a chemical waste treatment facility can result in serious injuries and property damage. This case study reconstructs the events leading to a fire and explosions during chemical waste treatment. It highlights the value of conducting simulations to study the behavior of liquids and shares investigative challenges and limitations.

Treatment of a batch of chemical waste in a chemical treatment facility can potentially cause unexpectedly triggered explosions and an intense fire that can potentially spread to an extensive area. This can also cause injuries and destruction of properties within the affected area and lead to significant damage and liability claims. What triggered the fire and explosions? Forensic experts were engaged to investigate their contributory factors and root cause.

A waste treatment company had routinely and uneventfully collected, treated, and incinerated hydrocarbon waste streams. On the day of the incident, the liquid waste was transported to the treatment facility and transferred into smaller containers. As part of the treatment, the employees involved were adding and mixing isopropyl alcohol/water into the waste liquid a short distance from the incinerator area when highly flammable fumes were released, and an explosion and a major fire occurred.

Experts were engaged at a late stage to determine the trigger for the explosion. As part of the work scope, it was also determined that it was necessary to consider whether the mixing process of the waste could have been a trigger. The challenges were not having first-hand information from direct examination of the incident site and not being able to analyze the residual waste remaining at the incident site. The experts were only able to rely on prior investigations conducted at the relevant time by third parties.

These prior investigations found that the waste liquid had been contaminated with isobutene. Based on the chemical composition of the waste mixture obtained, forensic experts conducted simulation experiments on: (1) the behavior of isobutene in the chemical waste mixture; and (2) the mixing process to study the physico-chemical behavior of the resultant liquid, chemical compatibility, and the effect of addition rate.

The findings were instrumental in reconstructing the events and identifying the likely contributory factors and root cause of the incident. A combination of factors was found to trigger the explosion and fires: chemical incompatibility, volatility, temperature differential, mixing in a blending container with a valve open to the atmosphere, displacement of ignitable headspace vapors from the blending container by the added chemical, accelerated addition of the chemical, and proximity to the incinerator. The simulation experiments provided evidence of pressure build-up and ejection of liquids from the container. The ignitable vapors from the liquids likely accumulated on the floor and were drawn through negative pressure to the nearby incinerator, resulting in ignition and flash-back to the containers of waste.

Besides reconstructing the pre-incident events and conditions, the experts proposed a safer approach when encountering out-of-the-ordinary waste so that companies can prevent a reoccurrence and improve their management system.

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### Fire and Explosion, Forensic Simulation, Chemical Waste Treatment

## B145 The Combined Extraction and Analysis of Trace Organic, Inorganic, and Peroxide Explosives Residues

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**Learning Overview:** The goal of this presentation is to introduce attendees to a validated method that includes: (1) the combined extraction of inorganic, organic, and peroxide explosives residues; (2) the processing of samples using Solid-Phase Extraction (SPE) cartridges; and (3) the screening of processed samples using Gas Chromatography/Electron Capture Detection (GC/ECD) and Ion Chromatography/Conductivity Detection (IC/CD).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a validated method for the combined recovery of inorganic and organic explosives residues, thus reducing the sample preparation typically required for the analysis of post-blast explosives residues.

Explosives residues can contain both inorganic and organic compounds that often require multiple extractions for analysis by different methods. These residues are usually extracted separately using various solvents. Currently, collection swabs containing suspected explosives residues are cut in half and processed through two syringe extractions: water for inorganic species and acetone for organic compounds. The resulting extracts are then screened by IC/CD and GC/ECD. The splitting of the collection matrix increases processing time and decreases the amount of explosives residue that will be extracted for analysis by each method and thus, may lead to negative findings if the levels fall below the instrument's Limit Of Detection (LOD).

This presentation will cover the validation of a method for the combined extraction of both inorganic and organic compounds from a single Cotton Ball (CB) or Cotton-tipped Applicator (CA). The organic explosives analyzed included nitramines, nitroesters, nitroalkanes, nitroaromatics, and peroxides. The anions analyzed were chlorate, chloride, nitrate, nitrite, perchlorate, sulfate, and thiocyanate. The cations analyzed were ammonium, barium, calcium, lithium, magnesium, potassium, sodium, and strontium. The method includes extraction in a water:acetone solvent followed by SPE, resulting in a load fraction and acetone eluate that are analyzed by IC/CD and GC/ECD, respectively. The validation experiments completed were the determination of LOD, the evaluation of sample stability at two concentrations, and the assessment of possible interferences from collection matrices, improvised explosive device components, or environmental factors.

In terms of organic explosives LODs, all were detectable at 500ppb for both matrices, with some present at lower concentrations. The peroxides were detectable at 5ppm for both matrices, with one at 1ppm. The anions spiked on CBs were all detectable at 1ppm, with some detectable at lower concentrations. For CAs, although some anions yielded detectable responses at lower concentrations, all were detected at 1ppm with one exception. Chloride failed due to a high matrix blank response. Cations were all detected on CB at 1ppm (with some at lower concentrations), but sodium and potassium failed with CAs due to a high matrix blank response.

For stability experiments, in the case of CB organics, 6 out of 12 organic explosives were considered stable up to 72 hours for the low concentration and 9 out of 12 explosives for the high concentration. In the case of CA organics, 3 out of 12 organic explosives were considered stable up to 72 hours for the low concentration and 8 out of 12 explosives for the high concentration. Hexamethylene Triperoxide Diamine (HMTD) CBs remained stable up to 32 hours for the low concentration and 16 hours for the high concentration. HMTD CAs remained stable up to 8 hours. Triacetone Peroxide (TATP), cations, and anions were considered stable up to 72 hours for CBs at all concentrations.

A large number of relevant matrices (65) were analyzed for their potential interference in the detection of analytes. It was determined that 28 of the samples interfered with organic and peroxide analytes. For the anions and cations, it was determined that 24 and 35 samples contained interferences, respectively. The analytes with the highest number of interferences were cyclotetramethylene tetranitramine (HMX), chloride, perchlorate, and potassium. Recovery experiments were also completed for swabs that were spiked with used motor oil (simulating contamination) and the current syringe protocol was compared with this validated SPE method.

In conclusion, a method for the combined extraction of inorganic and organic explosives residues was validated. The LODs and stability of analytes were determined, and 65 interference matrices were analyzed. The described method permits the processing of a single swab for analysis by IC/CD and GC/ECD for a streamlined approach to trace detection of explosives residues.

### Explosives, Solid-Phase Extraction, Trace Analysis

## B146 The Optimization of Gas Chromatography-Vacuum Ultraviolet (GC-VUV) Spectroscopy for the Analysis of Explosives and Their Post-Blast Residues

*Courtney Cruse, BS\*, Indianapolis, IN 46217; John V. Goodpaster, PhD, FIS Program, Indianapolis, IN 46202*

**Learning Overview:** After attending this presentation, attendees will better understand the analysis of explosives via GC-VUV and the optimization of the system to improve detection levels for post-blast debris analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a recently developed detector for GC (a VUV spectrometer) and its optimization for the analysis of post-blast debris.

The instrumentation 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), hydrogen carrier gas (3.2mL/min flow rate), a Restek® Rtx®-5MS column (15m x 0.32mm ID x 0.25µm), and a VGA 101 VUV spectrometer. The VUV spectrometer was operated with nitrogen as the make-up gas (0.35psi), a transfer line/flow cell temperature range of 200°C to 300°C, and a spectral range of 125nm to 430nm. The optimization method utilized was a face centered central composite design, and the parameters that were optimized included the final inlet temperature (200°C, 250°C, 300°C), GC flow rate (1.9mL/min, 3.2mL/min, 4.5mL/min), and make-up gas pressure (0.00psi, 0.15psi, and 0.30psi). Flow cell temperature was optimized independently (200°C, 250°C, 300°C).

Various explosive compounds of interest were included in the optimization: Triacetone Triperoxide (TATP), 2,3-Dimethyl-2,3-Dinitrobutane (DMNB), Nitroglycerine (NG), diphenylamine, 2,4,6-TRinitrotoluene (TNT), Pentaerythritol Tetranitrate (PETN), and RDX. Nitrate ester and nitramine explosives thermally decompose in the transfer line/flow cell of the VUV detector at higher temperatures (>250°C) into nitric oxide, carbon monoxide, formaldehyde, oxygen, and water. Compounds that thermally decomposed (NG, PETN, and RDX) had higher optimized GC flow rates (4.5mL/min, 4.5mL/min, and 2.6mL/min, respectively), while the remaining compounds had an optimized flow rate of 1.9mL/min. All compounds had an optimized make-up gas pressure of 0.00psi and final inlet temperature of 200°C with the only exception being diphenylamine with an optimized temperature of 286°C.

To investigate the applicability to forensic post-blast debris analysis, a bomb squad assisted in obtaining realistic samples of post-blast residues of single-base (IMR 4064) and double-base (Alliant Red Dot) smokeless powder 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 original method and the optimized method for comparison.

### Gas Chromatography, Vacuum Ultraviolet Spectroscopy, Explosive Analysis

## **B147 A Comparison of Spectra for Common Dyes on Five Different Mounted and Unmounted Textile Fiber Types Using Raman Spectroscopy**

*Patrick M. Rydzak, PhD\*, Federal Bureau of Investigation Laboratory, Quantico, VA 22135; Kyleen E. Elwick, PhD, Federal Bureau of Investigation Laboratory, Quantico, VA 22135; James M. Robertson, PhD, RSU, Quantico, VA 22135*

**Learning Overview:** After attending this presentation, attendees will better understand how Raman spectroscopy can be used to identify dyes on commonly found textiles, what factors may interfere with proper identification, and methods that can be used to mitigate these factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a baseline for how Raman spectroscopy may be used in forensic work for the identification of various dye types and colors on several common textiles. As forensic work with fibers often involves mounting single, possibly faded, fibers on slides for ease of examination and long-term storage, this presentation will provide attendees with information on how mounting media and relative dye concentration may affect the results of their analysis. Finally, this presentation will provide examiners with guidelines to help maximize the chances that their analysis may lead to positive identification of fiber dyes with Raman spectroscopy.

Raman spectroscopy is a commonly used technique in forensics that has been successfully employed in the identification of inks, paints, and textile dyes. The objective of this study was to compare the Raman spectra of commonly found textile dyes when dyed on their respective fiber in forensic settings. It was hypothesized that fading dye color and common standard operating practices such as mounting fibers onto glass slides with media may negatively impact the dye signal and investigative results. To test this, the spectra of four common categories of dyes (acid, reactive, disperse, and direct) were analyzed on five different textile fabrics. Each of these fiber types (nylon, wool, cotton, polyester, and viscose rayon), were dyed with four colors: black, blue, red, and yellow. Fibers dyed with blue, red, and yellow were dyed with three concentrations of dye (1%, 0.5%, and 0.05% w/w). Raman spectra were taken for each dyed fiber mounted on glass slides with Permout™ or Entellan® New mounting media, as well as unmounted. Each spectrum was then compared to dye and fiber reference standards to determine the optimal parameters to detect the dye and the relative influence of different mounting media on dye spectra. After taking Raman spectra with two different lasers (532nm and 780nm) on the Thermo Scientific™ Nicolet™ Almega XR, it was determined that successful detection of dye spectra on dyed textiles was predicated on several variables.

Some variables noted to influence the successful detection of dye spectra included dye type, dye color, dye concentration, fiber type, laser source, laser power, microscope objective, the type of mounting media used, and post-measurement spectral processing. For example, it was determined that dye spectra of acid and direct dyes were more defined than spectra of reactive and disperse dyes, and therefore more likely to be detected. Other noted trends included the increased likelihood of the detection of red and yellow dyes over blue and black dyes, and the overlap of some fiber spectra with the spectra of their corresponding dyes, such as nylon and acid dyes. When mounting fibers, choice of mounting media usually did not influence dye detection. However, it was determined that direct blue and red dyes were more easily detected on rayon fibers mounted with Entellan® New than with Permout™. Depending on the fiber and dye, post-sampling processing could sometimes improve dye signal and increase the likelihood of detection, such as in the case of acid dyes when the wool fiber standard and mounting media background signals were subtracted from the spectrum.

To date, there are thousands of known dyes, dye mixtures, and textile blends currently on the market, as well as the different manufacturers of Raman instruments and wavelengths of lasers available. Considering this, it was not possible from the results of this study to make definitive conclusions on recommendations for detecting dye spectra from unknown samples. However, based on the type of fiber being examined and the color of the dye, there are general guidelines presented here that may maximize the likelihood of positive identification, particularly in cases where fibers are mounted onto slides for examination. There is currently a need for further studies utilizing other Raman instrumentation, analysis software, and laser wavelengths to determine the optimal parameters for the detection of dyes on textile fibers.

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### **Raman Spectroscopy, Dyes, Textiles**

## B148 A Population Study of Textile Fibers on Parapets of High-Rise Housing in Singapore

Sherni Koh, BS, Health Sciences Authority, Singapore 169078, SINGAPORE; Vanessa Eng\*, Health Sciences Authority, Singapore 169078, SINGAPORE

**Learning Overview:** After attending this presentation, attendees will have gained insight into the background fiber population in the Southeast Asian country of Singapore.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the first fiber population study done in a Southeast Asian country, characterized by a typical tropical climate with abundant rainfall and high humidity all year round. This study seeks to compare to some of the previous population studies and investigate if geographical and climatic changes present any effect on background fiber population.

Singapore is one of the most densely populated countries in the world and more than 80% of the population stay in high-rise housing.<sup>1</sup> As a result, fall-from-height deaths are common in Singapore. Sometimes, it is crucial to determine where the fall started. The presence of transferred fibers onto the parapets of housing estates could provide key evidence in such cases. However, the significance of such fiber evidence would be difficult to assess without any background fiber statistics.

Fibers were lifted from the surfaces of the parapets from the ninth and tenth levels of ten housing locations. The microscopic characteristics and optical properties of the fibers were examined and compared using polarized light microscopy and fluorescence microscopy techniques. The frequency of fibers recovered from these surfaces were ranked in terms of color and generic fiber type. The fibers recovered from the parapets at each level and location were compared against each other.

A total of 1,256 fibers were recovered from ten housing locations. They ranged from having a minimum of 25 fibers to a maximum of 148 fibers at each level, with a mean of 63 fibers. No trends were observed in the number of fibers recovered with respect to the level or the location. Gray/black, blue, colorless, and red were the four most abundant colors that were present in the population while cotton, polyester, and rayon fibers were the three most abundant generic types. With a combination of color and generic type, blue cotton, gray/black cotton, and colorless polyesters were the three most abundant fiber types that were present in the population.

Thirteen out of 20 levels examined were found to have similar fibers within the same level. In a total of ten locations, only two locations had similar fibers between levels nine and ten.

### Reference(s):

- <sup>1</sup>. *Singapore Housing & Development Board Annual Report 2018/2019*, Key Statistics, Page 13. <https://services2.hdb.gov.sg/ebook/AR2019-keystats/html5/index.html?&locale=ENG&pn=1>.

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### Population Study, Fibers, Singapore

## B149 The Selection and Spatial Analysis of USDA-NRCS Soil Survey Map Units Based on Typical Soil Color for Forensic Investigations

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**Learning Overview:** The goal of this presentation is to review the goals and approaches of forensic soil examinations and present a new reference data set that may be used in geographic interpretation of soil color. This presentation will describe the nature and structure of the United States Department of Agriculture-Nature Resource Conservation Service (USDA-NRCS) National Cooperative Soil Survey (NCSS) data and how it can be judiciously applied in a soil provenance investigation, possibly to provide context to forensic soil comparison

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining the fact that soil color is very commonly considered in forensic soil examinations as it is easily observed, non-destructive, and commonly discriminating. However, prior to recent advancements, linking color of evidentiary soils to soil maps to aid in a forensic soil provenance interpretation was exceeding tedious because color is a text field in the official series descriptions. The recently digitized soil color data now enables color to be more easily used as part of a soil provenance interpretation and to possibly provide context for the rarity of a soil color within an area of interest.

Soil color is commonly observed early in the examination scheme of forensic soil traces and has value in soil comparison and in soil provenance examinations in which the goal is provide leads for the likely geographic source of the material. The NCSS provides detailed soil surveys for most of the country, representing the spatial arrangement of soils at scales from 1:12,000 to 1:63,000. Typically, each soil map unit (the basic unit of spatial delineation) contains information associated with one or more soil series concepts.<sup>1</sup> This database lacks soil color information, but reasonable selections of representative color data are documented in the Official Soil Series Descriptions (OSDs). Typical moist and dry soil colors were extracted from the OSDs (roughly 26,000 documents) and arranged into a new database containing Munsell colors as Hue, Value, and Chroma, as well as the calculated RGB and L\*a\*b\* color parameters.<sup>2</sup> This creation of this database has enabled the linking of soil color data to the detailed Soil Survey Geographic (SSURGO) database via soil series name, thus the creation of query-able soil color maps. The resultant soil color maps permit selection of map units by the color of either the dominant component or of any constituent soil series within each map unit. These query-able soil color maps have limitations and uncertainties due to the natural variability found in the source data and the interpolations made in creation of soils maps, but provided that the users of these maps understand their inherent limitations, these soil color maps may have utility in forensic soil reports or other applications.

The new availability of query-able soil color maps enables spatial analysis and integration with geological, botanical, and infrastructure maps in the interpretation phase of a forensic soil provenance examination. An example of a derived soil color map that was used to aid in the successful search for a body based on soil evidence will be presented, with identifying case details withheld.

Soil color maps of an area of interest provide context for soil comparisons by objectively representing color prevalence and have been applied to both emphasize rarity or commonness of a particular soil color. In one case, soil evidence was purported to be derived from the infield of a baseball diamond. The color of this synthetic soil was absent from any of the soil series mapped within the county. Likewise, soil survey data has been cited for how spatially extensive soil with similar properties, including color, are within an area to de-emphasize the significance of failing to exclude a common source.

### Reference(s):

1. Soil Survey Staff, Nature Resource Conservation Service, United States Department of Agriculture, *Soil Survey Geographic (SSURGO)* Database. Available online.
2. *Soil Colors of the Continental United States*. <https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/geo/?cid=nrcseprd1423827> and <https://nrcs.app.box.com/v/soils/folder/53525984812>.

### Soil Color, Soil Survey, GIS

## B150 Modern Single Grain Forensic Palynology: Preserving the Evidence for a Comprehensive Analysis

Luz J. Kelley, MS\*, National Center for Forensic Science, Orlando, FL 32816; Bryan McCullough, Orlando, FL 32826; Matthieu Baudelet, PhD, National Center for Forensic Science, Orlando, FL 32826

**Learning Overview:** After attending this presentation, attendees will have gained an understanding of a non-destructive genomic analysis application of single pollen grains by combining the techniques of quantitative Polymerase Chain Reaction (qPCR), DNA sequencing, and microscopy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simplified and quantitative pollen grain analysis by combining two commonly practiced protocols that can be utilized in forensic labs at the state or local levels.

Palynology, the study of pollen grains, is known to play an essential role in forensic casework. Forensic palynology uses pollen as evidence to link an object or person to a particular time or place and has played a crucial role in many criminal investigations, from burglary to homicide, rape, war crimes, terrorism, and drug trafficking.

The identification of plant species from their pollen relies on two traditional methods: microscopy or genetic analysis. On the one hand, microscopy relies on identifying pollen grains through morphology (i.e., size, shape, and wall structure) and comparing it to an image library for accurate identification. On the other hand, genetic analysis characterizes pollen species using a short DNA sequence from a universal standard in the genome. Both methods have so far been mutually exclusive. The standard procedure for microscopy is to clean the grain through acetolysis, which destroys any genetic material in the sample. Studies involving genomic characterization of the plant material requires the release of the genomic material by mechanically crushing the grain, which can no longer be analyzed for morphology through microscopic methods. While the number of forensic palynological studies increases, they usually rely on only one of the two techniques above and rarely show the potential for an efficient analysis of individual grains within an assemblage to statistically evaluate the species distribution in the mixture of grains that can be the evidence.

This study considers that for forensic analysis, a non-destructive method for such evidence at the single grain level is essential. Keeping the evidence intact after DNA analysis allows secondary analysis in the future by microscopy if needed. Furthermore, the ability to perform genomic analysis for each pollen grain of the evidence bypasses the issue of DNA mixtures more likely to be observed in a pollen assemblage. To respond to this need, a new method was developed that does not destroy the pollen grain and allows for the extraction of genetic material on a single grain level.

In this study, *Pinus echinata* (shortleaf pine), *Taxodium distichum* (bald cypress), and *Plantago lanceolata* (ribwort plantain) individual pollen grains were used to demonstrate this approach, with qPCR of the chloroplast genetic marker *rbcLw* and the second nuclear ribosomal ITS2 region, DNA sequencing, and digital microscopy, confirming the non-destruction of the pollen grains. The DNA obtained non-destructively from single grains opens the door for other downstream applications, including plant species identification at the individual grain level in samples such as soil and clothing swabs.

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### DNA, Pollen, Palynology



## **B151 Forensic Paint Analysis With Simultaneous Optical Photothermal Infrared (O-PTIR) and Raman Microspectroscopy**

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**Learning Overview:** After attending this presentation, attendees will understand the advantages of using simultaneous O-PTIR and Raman microspectroscopy for the analysis of automotive paint. In addition, attendees will gain understanding of how O-PTIR works and the benefits it can provide the forensic science community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how simultaneous O-PTIR and Raman microspectroscopy can be used to obtain quality chemical information about each of the layers in automotive paint samples non-destructively, rapidly, and with submicron spatial resolutions.

Paint is a common type of trace examined in forensic laboratories because it provides valuable associative information for crimes involving vehicular accidents and home invasions. The complex chemistry of paint can be thoroughly interrogated with vibrational microspectroscopy, with results that are used for its classification and comparison. Traditional IR microspectroscopy is frequently used by forensic paint examiners to identify binders, some pigments, and additives, while Raman microspectroscopy affords the additional ability to identify colored pigments, thus providing complimentary information that adds to the complete chemical characterization of a paint sample. However, both traditional IR and Raman microspectroscopy have limitations. IR microspectroscopy has limited spatial resolution (10–20 $\mu$ m at longer IR wavelengths). Sample preparation for transmission IR microspectroscopy requires skill, is potentially complicated, and is semi-destructive and, while IR analysis with an Attenuated Total Reflection (ATR) objective is an option, it requires intimate contact with the sample (risking cross contamination, sample damage, or even damage to the ATR internal reflection element) and targeting a specific location can be difficult. Fluorescence, which is laser wavelength-dependent and sample specific, is the major limitation of Raman microspectroscopy.

The mIRage+R IR and Raman microscope is a new technology that combines O-PTIR with Raman microspectroscopy to enable the complementary and confirmatory analysis of a sample at the same time, same spot, and same spatial resolution. O-PTIR uses a visible light probe to measure the photothermal response of the absorption of radiation from a pulsed IR laser focused on the sample. The use of visible light allows for non-contact, reflection mode analysis with submicron spatial resolution that is independent of the IR wavelength. Further, analysis is fast (~1 second) and requires little to no sample preparation. The reflection O-PTIR spectra are free of specular and diffuse scatter artifacts and can be directly compared to commercial/custom transmission IR databases. Since this is combined with a Raman spectrometer, IR and Raman spectra are able to be simultaneously and non-destructively collected from a submicron-sized spot in seconds, create a line array in under a minute, and map an area of a sample to create a hyperspectral image within a few minutes to a few hours (depending on the size of the area and desired spatial resolution).

A variety of automotive paints which differ in color (red, blue, and silver), properties (number and chemistry of layers), and production year were collected from auto body shops in the Northeast United States and subsequently analyzed simultaneously with O-PTIR and Raman spectroscopy. For comparison, the samples were also analyzed with stereomicroscopy, brightfield microscopy, polarized light microscopy, traditional ATR IR microspectroscopy, and Raman Microscopy (with a 780nm laser excitation source). O-PTIR plus Raman microspectroscopy provided chemical information (e.g., binder and pigment identification) from all paint layers non-destructively, rapidly, and with minimal sample preparation. The submicron spatial resolution of O-PTIR enabled chemical analysis of paint layers with thicknesses less than 10 $\mu$ m. Hyperspectral imaging with sub-micron spatial resolution revealed chemical mixing between some paint layers at their interface, which has not previously been detected and has the potential to be used for forensic discrimination. This presentation will introduce the forensic science community to the benefits of O-PTIR combined with Raman microspectroscopy for the analysis of automotive paint.

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### **Paint, O-PTIR, Raman**



## **B152 A Standard Development for Visual Color Determination and Comparison in Forensic Soil Examinations: The Organization of Scientific Area Committees (OSAC) Subcommittee on Geological Materials**

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**Learning Overview:** The goal of this presentation is to describe the goals, justification, and purpose for the development of a standard for visual soil color determination as it applies to forensic soil examinations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how the provision of standards for visual soil color determination and criteria for exclusion in forensic comparisons will provide the means for uniform processes to be employed by forensic laboratories as well as guidance in forensic soil examinations for professional geologists or soil scientists who may be asked to provide expertise to law enforcement.

Upon inception of the OSAC for Forensic Science, a geological materials subcommittee (OSAC-GEO, now a group within OSAC-Trace) has been developing documents to improve the analysis and interpretation of geological evidence as part of *criminal* investigations. This mission scope contrasts with *environmental* soil forensics, in which methods focus on detecting environmental contaminants or on the role of soil in civil engineering, for which distinct standards are needed. OSAC-GEO primarily focuses on analysis of geological materials as trace evidence that can be examined to provide information about how or where a putative criminal act occurred.

Among the initial documents produced by OSAC-GEO is one focused on visual soil color characterization and is currently in the process of pursuing approval as an American Society for Testing and Materials (ASTM) standard. Because visual examination of soil color is fast and non-destructive, it is typically characterized during the initial screening of forensic specimens; within the context of a soil comparison, color can be suitable for excluding a common source or directing further orthogonal analyses (e.g., microscopy and elemental analysis, among others). Because color is used as a screening metric, the criteria proposed for soil color comparisons are intended to minimize false exclusions and the “inclusion” windows are relatively coarse. Soil color may also be useful in soil examinations aimed at constraining the possible geographic source(s) of the soil to aid in investigative casework.

This document for forensic soil color determination is restricted to visual evaluation by reference to a soil color chart in the Munsell color system. While instrumental colorimetry can provide better precision, the proposed comparison criteria within the OSAC-GEO-developed document are specific to the discrete nature of pigmented chips in the soil color reference books widely used in the soil science community.

This document on soil color also describes circumstances in which color should not be used as a part of a forensic soil comparison, including: alteration or contamination of any of the samples being compared; non-representative soil exemplars; distinct treatments or size fractionation among the comparators; or when soils have different moisture contents.

The comparison criteria for forensic soil comparisons build upon the methods developed by the United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) -National Cooperative Soil Survey for describing soil color contrast within a soil horizon. Specifically, boundaries between *faint* and *distinct* color contrast are expanded slightly in the case of low chroma soils. These color contrast thresholds are employed as the criteria to determine if two or more soils could share a common origin. If the soils exhibit colors with “faint” contrast, then they may have a common origin and further analytical characterization of the specimens is required. If the color contrast between two soils exceeds the exclusion criteria, then the analyst may conclude the soils originated from distinct sources.

Proposed color contrast criteria for inclusion in a forensic soil comparison are: (1) both soils are dark (values  $\leq 3$ ) with low color saturation (chroma  $\leq 2$ ); (2) both soils have low color saturation (chroma  $< 2$ ), have the same values/lightness and have hue offsets of  $< 7.5$  hue units; or (3) the soil colors are offset by  $\leq 1$  chip in hue, value, and chroma in a standard Munsell Soil Color Book. Criteria 1 and 3 are derived directly from the boundary between faint and distinct color contrast defined by the NRCS-National Cooperative Soil Survey.

As color is a screening method, a color-based provisional inclusion must be followed by orthogonal higher discrimination examination methods; however, color may serve as a sufficient exclusionary difference in a soil comparison.

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### **Soil Color, OSAC, SDO**

## **B153 Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS): Method Optimization**

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**Learning Overview:** After attending this presentation, attendees will understand the value PCRS holds in forensic soil analysis. The optimal soil mineral parameters for automated imaging, Raman analysis, and dispersion will be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by completing the first steps in achieving the overall aim of developing a robust, automated, and objective analytical method for the analysis and comparison of soil minerals using PCRS.

Soil is a valuable and powerful trace evidence that provides linkages and investigative leads. Criticisms of forensic soil analysis (e.g., subjective, labor-intensive, time-consuming) have resulted in a considerable decline in its use in forensic investigations and created countless missed opportunities within the criminal justice community. Consequently, there is a need for a statistically supported, automated, and objective analytical method for soil analysis. PCRS, also known as particle driven or Morphologically Directed Raman spectroscopy (MDRS), is a novel yet reliable analytical technique that can add significant value to the forensic examination of soil evidence. It has proven easy to use and non-destructive, and its ability to be automated enables a large amount of data to be collected with minimal time required by the criminalist. Resulting data provides qualitative and quantitative information on a sample. PCRS obtains particle images of soil components (i.e., mineral) and produces microscopic morphological characteristics (e.g., circularity, elongation, brightness) and particle size distribution for the particles present. Particles can then be targeted randomly or based on morphological characteristics for Raman analysis to provide secure mineral identification.

The research presented here focuses on PCRS method optimization for soil mineral analysis using traditional figures of merit and response surface modeling of a multi-level experimental design. Laser wavelength, laser power, magnification, grating, and exposure time were examined for chemical identification of minerals via Raman spectroscopy. Particle size, particle destruction, and detector oversaturation influenced the final parameters to be evaluated for spectral analysis. The optimal parameters were determined based on a complex mineral mixture that reflects the diversity of minerals (based on stability, structure, Raman scattering capability, transparency, and fluorescence) that may be found in a single soil sample. The contrast/illumination method, magnification, and targeted morphological analysis were then evaluated to obtain an optimized method for automated imaging and Raman analysis. The effect of field of view and contrast on particle sensitivity and time of acquisition were important factors considered. At this step, final parameters were set for each soil type based on the individual parameters determined necessary for minerals of that given soil type. Now that the optimal method parameters have been determined, they can be applied to the analysis of a range of soil samples to generate a robust PCRS dataset for forensic soil analysis.

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### **Forensic Soil Analysis, Raman Microscopy, Minerals**

## **B154 The Scent of Death: Analyzing the Molecules of Decomposition and Influence on Insect Succession via Solid Phase Microextraction (SPME)**

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**Learning Overview:** After attending this presentation, attendees will develop a better understanding of thanatochemistry (or the chemistry of death), the Volatile Organic Compounds (VOCs) that are released from decaying remains, and its relationship with insect succession. Additionally, attendees will learn more about the human decomposition process and how analytical techniques were used to collect the emitted compounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a temporal evolution profile of the decompositional-related VOCs via the use of an easy method for detecting and quantifying the released compounds. Attendees will be provided with a correlation between insect succession and cadaveric VOCs.

Decomposition is the complex process in which chemical compounds are broken down into simpler organic or inorganic matter. The first stage of decay, or autolysis, begins immediately after death, which causes the body to become more acidic in nature due to the build-up of carbon dioxide. As the pH changes, cells begin to lose structural integrity, resulting in cell membranes rupturing, releasing enzymes that begin eating the body from the inside out.<sup>1</sup> Subsequently, VOCs begin to release. Despite the fact that there has been an increased interest in researching VOCs related to decomposition, there continues to be a lack of an in-depth cadaveric VOC profile. Decompositional-related VOCs can provide scientists with vital forensic information that can aid scientists in developing a portable device used for search and recovery of victims or dead human remains in police investigations. Due to an arthropod's life cycle and predictable sequence of entomofauna succession, insects provide a valuable tool, which may be used to estimate a Postmortem Interval (PMI) range.<sup>2</sup> Due to the highly nutritious source that human remains provide as well as the olfactory properties associated with decomposition, insects and other arthropods can colonize a body within five minutes of death.<sup>2</sup> The particular order of succession between different species is dependent on the stage of decay as well as the purpose of colonization. Though it is known that certain species of insects are attracted to the VOCs released by a corpse during different stages of decay, the specific chemicals each species is picking up on is still unknown. Therefore, it is important to determine if there is a correlation between emitted VOCs, stage of decomposition, and insect succession. In this study, the VOC temporal profile of pig (*Porcus*) carcasses as human proxies are used to determine if there is a correlation between the presence of specific chemical compounds or mixtures and insect succession.

Stage one of the study involved collecting, identifying, and quantifying VOCs emitted from decomposing biomedical swine carcasses. Collection was conducted using a fast and easy technique that incorporated Solid Phase Microextraction (SPME) fibers and the VOCs were further analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). The project focused on the examination of released compounds in a controlled, indoor environment as well as in an outdoor setting to determine whether or not insect activity conditions had any impact on the formation and distribution of the VOCs from the cadaver during the decomposition process. The data obtained was then compared to a previous study that evaluated the VOCs that were released during the outdoor decomposition process of human cadaver analogs. Differences, as well as similarities, in the compounds detected will be discussed in this presentation, as well as the impact that insects have on the release of VOCs from decomposing remains. Stage two entails preliminary studies on the relationship between released cadaveric VOCs and insect succession using fly traps. These traps contained a variety of released chemical compounds during decomposition placed for outdoor field testing in 48-hour increments to determine ecological correlation. After the allotted time period, the fly traps are collected in order to identify any trapped insects. It is predicated that there will be an association between the two factors, providing more insight into insect succession during decomposition.

### **Reference(s):**

1. Lee Goff, M. Early Post-Mortem Changes and Stages of Decomposition in Exposed Cadavers. *Experimental & Applied Acarology* 49, no. 1–2 (October 2009): 21–36. <https://doi.org/10.1007/s10493-009-9284-9>.
2. Verheggen, François, Katelynn A Perrault, Rudy Caparros Megido, Lena M Dubois, Frédéric Francis, Eric Haubruge, Shari L Forbes, Jean-François Focant, and Pierre-Hugues Stefanuto. The Odor of Death: An Overview of Current Knowledge on Characterization and Applications. *BioScience* 67, no. 7 (June 7, 2017): 600–613. <https://doi.org/10.1093/biosci/bix046>.

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### **Volatile Organic Compounds, Decomposition, Insect Succession**

## B155 A Review of the Scientific Foundations for Firearm Examination

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**Learning Overview:** After attending this presentation, attendees will be able to comprehend the wide range of results obtained for measurement of error rate in firearm examinations and the large number of influence factors on those results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling attendees to derive an improved understanding of the scientific bases underlying firearm examination, its current state of the art, and its future directions.

Forensic science plays a vital role in the criminal justice system by providing scientifically based information through the analysis of physical evidence. Several scientific advisory bodies have expressed the need for scientific foundation reviews of forensic science disciplines, and the National Institute of Standards and Technology (NIST) has been tasked as an appropriate agency for conducting them. A scientific foundation review is a study that seeks to document and evaluate the foundations of a scientific discipline; that is, the trusted and established knowledge that underpins the discipline's methods. These reviews seek to answer the question: "What empirical data exist to support the methods that forensic science practitioners use to analyze evidence?" The purpose of a scientific foundation review is to document and consolidate information supporting the methods used in forensic analysis and identify knowledge gaps where they exist. This presentation will enable attendees to comprehend the wide range of results obtained for measurement of error rate in firearm examination and the large number of influence factors on those results.

NIST began by developing a general specification for these reviews, then undertook its first one, a review of DNA mixtures, now nearly complete.<sup>1</sup> Subsequently, NIST initiated reviews of bitemark analysis and digital evidence.

The review of Scientific Foundations for Firearm Examination, begun in October 2019, is the fourth in the series. The project team is evaluating a wide body of published literature and other materials devoted to firearm examination. A bibliography database has been developed containing 690 references (as of October 2020). Among other topics, the review includes historical perspectives of the field and current methods in use, key takeaways and considerations for the field, and studies of the advanced methods of 3D acquisition and objective algorithmic analyses, but the primary emphasis is placed on the scientific foundations of comparison microscopy, which has been the most widely used method by far.

Toward this end, all known literature and materials that contain studies of error rates for identification and exclusion of matching bullets and cartridge cases have been included with a focus on 29 reports of interlab studies, method validations, and proficiency tests. As suggested in the PCAST report, these works may be classified into three categories: (1) "open, independent" studies such as that by Baldwin et al., wherein each question posed is independent of the others; (2) "open, dependent" studies, such as that by Smith et al., wherein each decision changes the *a priori* probability of other decisions; and (3) "closed" studies, wherein every questioned unknown has a true match in the set.<sup>2-4</sup>

In addition, six other factors are specified that influence the values of measured error rates. These include: (1) the region of interest (bullet land engraved area, breech face impression, etc.); (2) the study size (equal to the number of questions multiplied by the number of participants); (3) whether or not known pairs are included as examples and standards; (4) whether or not the study includes consecutively manufactured firearms; (5) whether questions are declared to participants or covertly included in case work; and (6) test difficulty.

The core team includes ten people, but others in the firearm examination community would be welcome to serve as a resource group of ideas and feedback to the team.

### Reference(s):

1. *NIST Scientific Foundation Reviews NISTIR 8225 Draft*. 2018, National Institute of Standards and Technology: Washington, DC. p. 31.
2. PCAST (President's Council of Advisors on Science and Technology). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*, Executive Office of the President, Editor. 2016, The White House: Washington, DC. p. 174.
3. Baldwin, D.P., et al. *A Study of False-Positive and False-Negative Error Rates in Cartridge Case Comparisons*. 2014, Defense Technical Information Center.
4. Smith, T.P., G.A. Smith, and J.B. Snipes. A Validation Study of Bullet and Cartridge Case Comparisons Using Samples Representative of Actual Casework. *Journal of Forensic Sciences* 3, 2016. 61(4): p. 939-946.

### Firearm, Examination, Foundation

## B156 Bricks Without Straw: Providing a Forensic Laboratory Experience in the Age of COVID-19

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**Learning Overview:** After attending this presentation, attendees will understand how to create virtual forensic science laboratories by relying on previously recorded data files and photomicrographs, as well as instructional videos posted online.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that meaningful forensic science laboratory exercises can be carried out online.

In the spring semester 2020, the COVID-19 pandemic forced the university to end in-person classes and to conduct all instruction online. The prohibition against in-person instruction continued in the fall 2020 semester. Several courses normally offered by the Department of Forensic Sciences in fall semesters have laboratory exercises. Several options were considered: deferring these courses to the spring 2021 semester; deferring the laboratories only to a later semester; or replacing the existing laboratories with virtual laboratories. The last option was adopted.

The virtual laboratories were required to be self-contained or to utilize material likely to be present in the home or easily acquired. The laboratories could make use of computer files acquired in previous laboratory exercises or files generated by course instructors especially for the course. Students could view videos showing sample preparation and instrument operation and then be presented with data and asked to interpret them.

FORS 6005 Fundamental of Forensic Science II focuses on laboratory disciplines such as the analysis of controlled substances, the analysis of trace evidence, and DNA profiling. The laboratory exercises for this course normally consist of a microscopy laboratory on the Becke line phenomenon and the identification of natural and man-made fibers, a gas chromatography/mass spectrometry laboratory on the identification of controlled substances, and a DNA-profiling laboratory in which the students obtain their own Short Tandem Repeat (STR) profiles. These laboratory exercises were replaced by the following virtual laboratories: (1) a Becke line laboratory using photomicrographs of glass particles mounted in immersion media with differing refractive indices; (2) a polarized light microscopy laboratory using photomicrographs of natural and man-made fibers (with crossed polars, and crossed polars with a first-order compensator); (3) a laboratory on the gas chromatography/mass spectrometry analysis of ignitable liquid residues using files from the National Center for Forensic Science (<http://ilrc.ucf.edu/>); and (4) a laboratory on the interpretation of DNA STR profiling using data files from previous classes.

FORS 6238 Forensic Chemistry I covers basic microscopy, forensic analysis of glass, and the forensic analysis of soil. The laboratory exercises in this course cover Köhler illumination, Becke line phenomenon, refractive index measurements, glass fractography, determination of soil colors, identification of clay minerals using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR), polarized light observation of interference figures, and the use of X-ray powder diffractometry to identify minerals. These were replaced with the following virtual laboratories: (1) a Becke line laboratory using photomicrographs of glass particles mounted in immersion media with differing refractive indices; (2) a glass fractography laboratory in which the students were required to perform fracture matches and to determine the direction of force that fractured a glass pane; (3) a soil color determination laboratory in which the students were given questioned and known soil samples and were required to compare their dried colors; (4) an ATR/FTIR laboratory in which the students were required to compare the spectrum of a questioned clay sample with a set of known spectra in order to find a match; and (5) two X-ray diffraction laboratories in which students were given data files for bulk soil samples and clay fraction samples and required to identify the minerals present in each.

Instructional videos of sample preparation and instrument operation were made available to students. The data files used were collected in previous years during laboratory exercises. The students were required to submit reports embodying their interpretation of the data presented in each exercise.

The virtual laboratory format permitted the instructor to meet the pedagogical goals for the courses, even in the absence of in-person laboratory instruction. This format excited greater student interest in the content of the laboratories as shown by the email exchanges about them between students and instructor.

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### Online Instruction, Virtual Laboratories, Forensic Science Education

## B157 A Characterization of Artificial Fingerprint Samples

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**Learning Overview:** The goal of this presentation is to build upon research surrounding artificial fingerprint samples and provide insight to using these samples for method development. Topics to be covered include reproducibility of DNA recovery from artificial fingerprint samples, ability to be visualized by several standard forensic imaging techniques, sample stability of both surface deposited and solution formats, and applicability and use on various challenging surfaces, including brass shell casings, wood, and tape.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further characterizing the biology and behavior of artificial fingerprint samples. These samples have value for touch sample method development by reducing the DNA variability associated with human latent prints and removing Institutional Review Board (IRB) -related complications related with collecting human-donated samples.

Assessment of DNA recovery from touch samples is difficult, as the initial amount of DNA deposited cannot be known and the amount of deposited DNA varies widely among individuals. This can make method development for touch samples challenging, and current approaches rely on the frequency of successful analysis and generation of complete profiles. However, these metrics do not enable a quantitative understanding of DNA recovery with a given method. To help overcome these challenges, this study developed artificial fingerprint samples containing a defined amount of single source human DNA in a sebaceous background. Previous work had established that the artificial fingerprint matrix mimic latent touch samples and lead to recoverable DNA from non-porous surfaces.<sup>1</sup> This study expanded on this prior work to further characterized artificial fingerprint samples, with the goal of evaluating artificial fingerprints as a broader touch sample method development tool.

This research performed several studies, including reproducibility of DNA recovery, ability to be imaged by standard forensic techniques, sample stability, and the extensibility to a variety of challenging substrates. Artificial fingerprint samples had reproducible DNA recoveries, in contrast to the range of DNA from human donor samples. As the initial DNA amount in the artificial fingerprint samples is known, this study determined the efficiency of the collection and extraction method, recovering ~8% of the total DNA deposited. To evaluate the ability to be imaged, artificial fingerprint samples were generated with ridge patterns and processed by conventional imaging techniques, including dusting, ninhydrin, and cyanoacrylate fuming with rhodamine 6G. Dusting powders were generally unsuccessful but both ninhydrin and cyanoacrylate fuming were able to adhere to artificial fingerprints and allow visualization.

This study wanted to understand the stability of the DNA in artificial fingerprint samples. Glass-deposited samples showed recoveries comparable to day one collected samples up to two weeks post-deposition under ambient storage conditions. Last, this study investigated the DNA recovery from more challenging substrates beyond glass, including wood, adhesive tape, and brass shell casings. DNA was recovered from all three surfaces with varying degrees of success. Brass required development of a deposition method that enabled DNA to be recoverable and not severely degraded. Assessment of chelators and various drying methods identified rapid drying under vacuum to most successfully preserve the DNA in the sample. Overall, these artificial fingerprint samples provide a reproducible test bed matrix for touch sample method development, may assist in evaluating methods that involve visualization, and can be compatible with a variety of surfaces for DNA recovery.

### Reference(s):

- <sup>1</sup> Danielle S. LeSassier et al. Artificial Fingerprints for Cross-Comparison of Forensic DNA and Protein Recovery Methods. ed. Andy T.Y. Lau, *PLOS ONE* 14, no. 10 (October 3, 2019): e0223170, <https://doi.org/10.1371/journal.pone.0223170>.

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### Artificial Fingerprints, Method Development, Latent Touch Samples



## **B158 Artificial Intelligence Applied to Image Quality Metrics of Latent Papillary Impressions**

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**WITHDRAWN**



## B159 A Quantitative Reliability Metric (QRM) for Querying Large Databases

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**Learning Overview:** The goal of this presentation is to showcase a novel measure of database search reliability. This is demonstrated for a Gas Chromatography/Mass Spectrometry (GC/MS) study of novel synthetic opioids in participation with the Houston Forensic Science Center.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that this metric gives a probability that an unknown object matches the closest reference objects returned from the database search. This has broad application because it can be used with any database and any comparison measure (e.g., distance or similarity). It generates a statistical probability of the quality of the match compared to a more similar matching object excluded from the database.

A modern tool for identifying data objects is by comparison to libraries of reference objects; in this case, it is mass spectra. Comparison metrics, such as correlation, are used to search for a list of the closest matching objects. In some instances, the correct identification is not the closest match because the object is not in the reference library or is distorted by noise; therefore, having a quantitative measure of the search reliability can help the analyst determine whether the queried data object is contained in the reference collection or finding the best match that may not be the closest match.

The QRM can be used with any database and any comparison measure. It was recently developed to assist in a National Institute of Justice (NIJ) - funded project for the identification of Novel Synthetic Opioids (NSOs) by GC/MS. A customized library of more than 223,000 mass spectra was queried with several thousand NSO samples. The QRM provided a statistical measure of the search quality but also proved invaluable for chromatographic peak detection, background removal, and library search optimization. The statistical probability compares the ordering of the closest matching reference spectra with the ordering of each matching reference spectrum when it is searched against the library. A variance can be calculated by the sum of the squared differences of the comparison metrics with the intra-library representing the ideal order. These variances are then compared to the variance that would be obtained if the correct matching spectrum was not in the library using the F-distribution.

The QRM is an independent measure of the quality of each library search result that can be used along with the comparison metric. The key advantage is that it can be used with any kind of library and any kind of similarity metric, and it provides a statistical result of the match reliability. For example, if the similarity measure is high and the QRM is low, then there is not much confidence in the result. However, when the similarity measure is low and the QRM is high, then the analyst can be confident in the identification. Last, the QRM provides greater contrast between the list of the closest matching reference objects than the comparison metrics.

Table 1. 4-ANPP (4-aminophenyl-1-phenethylpiperidine, CAS 21409-26-7) 0.02 ng injected

Hit Number	Similarity	QRM(%)	Name
1	0.717	92.7	4-ANPP
2	0.713	84.3	4-ANPP
3	0.705	84.1	4-ANPP
4	0.704	83.2	4-ANPP
5	0.698	82.8	4-ANPP
6	0.663	15.0	N-methyl-1-phenylcyclohexylamine
7	0.655	25.8	1-ethyl-6,7-dimethyl-2-indolinone
8	0.655	12.4	1,1-dideuterio-2-hydroxyethylcyclohexylcarbamate
9	0.654	10.0	3-butyl-5-methyl-1,8a-dihydroindolizine
10	0.652	3.3	3-methyl-5-N-butyl-1,2-benzisoxazole

**QRM, Quantitative Reliability Metric, Statistical Probability of Match**

## B160 Improving Our Knowledge of Drug Usage Through the Analysis of Used Injecting Paraphernalia

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**Learning Overview:** After attending this presentation, attendees will have learned from an innovative approach to gather objective data about illicit drugs. In particular, at the end of this presentation, attendees will have a better understanding of what drug types and other potentially harmful substances are present in the drug market and are used by people who inject drugs. Up-scaling and implementation of the approach would support early detection of potentially harmful substances entering drug markets (for example, highly potent fentanyl analogs). It would also provide authorities with more objective data on the issues surrounding illicit drug use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how existing common forensic science competencies (e.g., forensic chemistry, drug chemistry) can be expanded in a proactive and preventative space (in complement to the traditional reactive manner). This presentation will also have an impact on society as up-to-date laboratory data may better inform health messaging and support people who use drugs to make informed decisions, in line with harm minimization strategies.

Drug consumption, dependence on substances, and the illicit drug trade are significant challenges for most countries. Understanding such a complex and multifaceted issue requires a problem-orientated approach, gathering and comparing data from different sources (e.g., police statistics, chemical profiling, internet activity, self-report surveys). One relatively new source of data is the chemical analysis of residual drug content of used paraphernalia. Such analysis provides objective data about drugs, adulterants, and cutting agents injected by people who use drugs and is complimentary to more traditional data collection. Further, by studying patterns of drug injecting over time, the most effective policy response, including in harm reduction programs, can be adapted and updated based on consumption changes or newly detected substances. A pilot study involving the analysis of residual content of used injecting paraphernalia collected at a Supervised Injecting Facility (SIF) was undertaken in Sydney, Australia, in 2019.

The current work extends from the 2019 pilot study through 2020, including during the COVID-19 pandemic, at the same SIF, the Sydney Medically Supervised Injecting Centre (MSIC). It provides information on injecting drug trends and aims to provide an objective picture of the substances injected by consumers and add to existing methods of understanding patterns of use. By analyzing the substances injected by consumers through Gas Chromatography/Mass Spectrometry (GC/MS), chemical analysis of the discarded syringes, and comparing these results to the self-report, important messaging can ultimately be established, depending on how knowledgeable consumers/people who use drugs are regarding the substances they have injected.

For one week in early 2020, seven disposal bins containing all used injecting paraphernalia were collected from MSIC. The residual drug content of the 207 syringes was analyzed through a validated GC/MS method, and the main psychoactive substance and adulterants were described.

The most commonly detected drugs were heroin, methamphetamine, and oxycodone. Combinations of drugs, drug derivatives, and cutting agents were also seen. Results from laboratory analyses were consistent with self-reported drug usage. Variation over time was also examined. Up-to-date results, including the potential impact of the COVID-19 pandemic on drug consumption, will be provided during this presentation.

Such an approach is appropriate to be carried out regularly as it does not disrupt the usual running of the facility. By implementing longitudinal monitoring, further data regarding smaller time scale drug market changes can be developed, and the introduction of more potentially dangerous substances into the market can be more quickly identified. Combining these findings with data from other sources ultimately provides an enhanced capability to better understand and address the issue of illicit drug use.

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### Illicit Drugs, Used Syringes Analysis, Harm Reduction

## **B161 Heroin Identification by Fourier Transform Infrared/Attenuated Total Reflectance (FTIR/ATR) Spectroscopy on Petroleum Ether-Based Extracts**

*Roehl Marave Cinco, PhD\*, Oakland Police Department, Oakland, CA 94607*

**Learning Overview:** The goal of this presentation is to train analysts, chemists, and criminalists in efficient, effective ways to analyze suspected heroin samples through IR on petroleum ether (pet ether) -based extracts to reduce backlogs while maintaining high quality.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the feasibility of FTIR spectroscopy with ATR to identify heroin in pet ether-based extracts of suspected heroin street samples. By incorporating this IR spectroscopic technique into heroin analysis, drug analysts, criminalists, and chemists can complement or even replace Gas Chromatography/Mass Spectrometry (GC/MS) and microcrystalline methods for increased efficiency while maintaining quality and effectiveness. The impact of adopting this technique results in faster identification of heroin in complex mixtures and potentially lowers backlogs while achieving the highest possible selectivity through structural information.

Because heroin (diacetylmorphine) has been a major factor in the current opioid crisis and epidemic, this study has explored ways to supplement current analysis techniques for seized suspected heroin samples. Such seized samples represent a significant portion of cases and can present themselves in various forms of mixtures—as tar-like gummy or chunky material, brown powders of various shades, or off-white powders. For years, pet ether-based extracts have been used for microcrystalline testing to identify heroin, in conjunction with color testing. Acid/base chemistry is used along with the selective solubility in hydrocarbon solvent to achieve separation of heroin (base) from other components for microcrystalline testing. Moreover, this study has also explored ways to reduce potential exposure to hazardous chemicals and ease the production of reviewable data.

To build on the microcrystalline approach using a Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Category B technique, it was logical to examine the pet ether extracts using FTIR/ATR, a SWGDRUG Category A instrumental technique. Starting from about 50mg of seized drug mixture, the process of the base extraction with sodium carbonate solution and isolation with pet ether is outlined, followed by drying of the pet ether with sodium sulfate, then air evaporation of the solvent. The extraction takes around five to ten minutes, followed by FTIR/ATR data collection on the remaining dried solid for one minute. By contrast, GC/MS analysis data collection could take 30–40 minutes, including the blank run before the sample. After the analysis of 200 casework samples, a summary of the results from IR spectroscopy is presented and supported by GC/MS data. In over 90% of the samples, the resulting FTIR spectrum could be identified as heroin (base). For about 7% of the samples, the FTIR spectrum was less clear and could only indicate the presence of heroin. The advantages of the whole procedure are listed (ease, efficiency, confirmatory results, and applicability for all types of samples), along with some limitations and caveats (required sample size greater than 100mg, insufficient data for multiple drugs, and no inference to original salt form). Overall, the results show that the FTIR/ATR technique on pet ether-based extracts is a feasible, efficient, and effective tool for routine heroin identification in seized street samples.

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### **Heroin, FTIR, Petroleum Ether**



## **B162 Methods to Detect Changes in Illicit Product Markets From Routine Forensic Casework**

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**Learning Overview:** After attending this presentation, attendees will understand techniques to collect, monitor, and maintain surveillance datasets from routine forensic reporting workflows, as well as methods to detect changes in the underlying supply of illicit products in their local jurisdictions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework in which routine forensic casework can be organized into a single data stream, delivering a timely resource for investigators and their stakeholders. Illicit Product Surveillance (IPS), meaning the aggregation of per-case forensic analysis of illicit product characteristics and contents, provides a valuable source of data on local and regional trends in pathological behavior and subsequent risk to the public. IPS methodology has application to any forensic discipline involving the analysis of products exchanged in a loosely defined market system.

This presentation begins with a study of the mechanism by which forensic casework relates to the underlying distribution of drug contents in a hypothetical District of Columbia (DC) drug market. Methods for controlling for motivated sampling patterns by evidence collectors are discussed, and a simulation study is conducted to demonstrate advantages to the consideration of confounding covariates introduced by routine policing.

Demonstrative IPS studies using de-identified data are presented. Three products are selected for their diversity, prototypical features, and impacts to public health: synthetic opioid powders containing fentanyl, loose plant-like material containing Synthetic Cannabimimetic Agents ([SCA], commonly referred to as “Spice”), and crystalline solids containing synthetic cathinones (commonly referred to as “Bath Salts”).

A geospatial analysis detailing fentanyl’s transition from heroin adulterant to stand-alone powder product is conducted. A theory emerges, relying on unique combinations of demand-side forces and supply-chain pressures present in the synthetic opioid market. This theory is then applied to the initial detection and subsequent trends in illicit fentanyl pills, formed to look like legitimate oxycodone pills. Techniques to establish geospatial differentiation along product-composition zonal boundaries are discussed using the chemical composition of SCA exhibits. The detection of Phencyclidine (PCP) and SCA co-confirmations is then analyzed as a product-use-behavior change. Spatiotemporal zones of most-likely emergence are estimated from IPS of SCA and PCP and are shown to accurately describe the observed emergence zone. Initial threat assessment and surge analysis methods are presented using the example of crystalline solids containing eutylone, a synthetic cathinone.

This case study is generalizable beyond forensic chemistry data. An IPS analysis cycle for forensic products is outlined, providing a discipline-independent framework to guide inference from IPS data streams to their constituent supply populations. This presentation ends with a step-by-step guide to developing IPS capability at forensic laboratories of various resource categories.

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**Forensic Intelligence, Data Analysis, Forensic Chemistry**

## C1 A Study of Video Conferencing Software From an Authentication Perspective

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**Learning Overview:** After attending this presentation, attendees will better understand the capabilities, specifications, file structures, and metadata pertaining to video conferencing software, which will aid authentication examinations of video captures relating to such.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing detailed authentication information for commonly used conference-recording software. This is a growing, impactful, and current area of concern.

As highlighted by the COVID-19 pandemic, video conferencing software has become an essential tool for maintaining communication between groups of people, whether that relates to a workplace environment or friends and family. It is common that these programs allow for recording of the video and audio streams, sometimes in multiple formats. Naturally, the recordings taken using conference software are also susceptible to the same issues as other digital recordings, namely the potential for manipulations. When performing authentication examinations, it is critical that examiners have an understanding of the capabilities of the purported capture device/software, as well as other factors such as the file structures of original and edited recordings made with said devices.<sup>1</sup> For instance, if a recording in a standalone audio format is purported to be an original capture using Microsoft® Teams, knowing that this platform only records video, regardless of whether a video or voice call is made, would support the proposition that the recording is not an original. In the majority of cases, the examiner would have to create and/or maintain a database of multiple exemplar recordings in order to determine these factors, which can be time consuming.<sup>2</sup> In cases where this is not possible as the identity of the capture device is not known, multiple exemplar recordings from a variety of video conferencing software are required in an attempt to identify or eliminate the source, which can be even more time consuming than the first scenario. To address this issue, a large-scale study of the following was performed that included: (1) capabilities of the most common video conferencing software on the market today, including Zoom®, Microsoft® Teams, and Skype® running on a variety of operating systems and devices, including Windows®, OSX®, Android™, and iOS®; (2) the specifications of the captured recordings from each of these software; (3) the file structure of recordings captured from each of these software; and (4) the Exchangeable Image File Format (EXIF) data fields and information captured from each of these devices.<sup>3,4</sup>

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### Video Authentication, Metadata, Video Conferencing

## C2 Houston, We Have a Problem: Deepfake Is the Word!

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**Learning Overview:** After attending this presentation, attendees will be familiar with various techniques of Deepfake creation and forensic detection. The main techniques are currently based on statistical analysis of Deepfakes coming from specific architectures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by attempting to present all the existing solutions as of today with a simple but schematic view. All the techniques will be presented organized in various categories and the corresponding pros and cons will be exposed, considering practical application scenarios.

Multimedia contents created by means of Artificial Intelligence (AI) techniques are introducing several big problems in the multimedia forensics field with the birth of the Deepfake phenomenon. Images and videos can be created through models like Generative Adversarial Network (GAN) where it is possible to create or swap a person's face from input source to target data.<sup>1</sup> Various state-of-the-art approaches are available to create AI-synthetic data, including film, photography, video game, and virtual reality productions. Politicians, actors, and famous people are the main targets of this new phenomenon: a famous example was the video of the former Italian premier, Matteo Renzi, created by a satirical newscast in September 2019, in which Renzi talked about his colleagues in a "not very respectful" way. Another famous example was the video of Mark Zuckerberg in which he claimed a series of statements about the ability of his platform (Facebook®) to steal data of its users. However, GANs are the new weapons as they can be used to create pornographic Deepfakes, raising a serious security threat. It is a fact that 96% of Deepfakes on the internet are pornographic. This threat needs to be mitigated with new forensic detection techniques capable of identifying whether multimedia data is deepfake, (i.e., created/alterd through/by GANs). This isn't a new problem; in fact, image manipulation was born almost immediately after the advent of photography, and currently, through the use of image editing tools (e.g., Adobe® Photoshop®, After Effects® Pro and GNU Image Manipulation Program [GIMP]) it is very easy to perform a forgery operation. Using conventional signal processing tools, images can be easily edited to achieve realistic results that can deceive even a careful observer. Various studies show that a digital image or video (without any kind of manipulation) contains within them different characteristics that allow the acquisition device to be uniquely identified. To detect Deepfakes, different methods have already been presented, many of them based on complex neural network solutions, and claim to obtain excellent classification results, but unfortunately it has been shown that they are not generalizable.<sup>2</sup> It was recently discovered that all GANs leave a fingerprint in the generated images that characterize the generative deep architecture. By exploiting these unique traces, it has been shown that it is possible to distinguish not only if the data is a Deepfake, but also reconstruct, with high precision, the type of architecture employed.

### Reference(s):

1. Goodfellow, Ian et al. Generative adversarial nets. *Advances in neural information processing systems*. 2014.
2. Hulzebosch, Nils, Sarah Ibrahim, and Marcel Worring. Detecting CNN-Generated Facial Images in Real-World Scenarios. *Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition Workshops*. 2020.

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### Deepfake, Multimedia Forensics, Generative Adversarial Network

## C3 The Recreation and Visualization of Runtime Objects Relationship From Process Memory Images

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**Learning Overview:** After attending this presentation, attendees will have gained an understanding of an app-agnostic technique for the recreation and visualization of process execution paths and the in-memory object relationship network. The goal of this presentation is to illustrate how these relationships can aid cybercrime investigations and malware analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating a novel effort in userland memory analysis that aids investigators in piecing together the context of valuable forensics evidence to determine its provenance, execution path, and overall scope within the application trace. Similar to the field of archaeology, where historical human activities are determined by recovering and piecing together material from a culture, this presentation will demonstrate the reconstruction of object relationships from process memory images without prior knowledge of the application semantics.

As userland memory forensics continues to be a practical and crucial alternative to kernel-level memory forensics and traditional disk forensics in program analysis and cybercrime investigations, there is a need to develop techniques that go beyond simple data recovery. Specifically, more sophisticated semantic analysis capabilities that reconstruct the state of a system under investigation from the volatile memory image are needed. In recent years, various application-specific memory analysis techniques that recover forensically interesting artifacts from well-known applications such as Facebook®, default messaging apps, and Telegram®, were presented in the literature.<sup>1-5</sup> Although these techniques are useful and often adopted by practitioners to provide forensics evidence, their methodologies are conceived based on an individual app's specific logic. Hence, their resulting recovery algorithm cannot be generalized to other applications or even different versions of the same app. Thus, this research presents a post-execution and app-agnostic semantic analysis approach designed to help investigators establish concrete evidence by exploring application execution paths and recreating the relationships between in-memory objects in a process memory image. The technique utilizes DroidScraper to find all the objects allocated in the process heap.<sup>6</sup> Then, treating each object as a node, it utilizes Heap Context Points-to analysis to establish the graph edges (representing the relationships between the graph nodes). In Heap Context Points-to analysis, object relationships are determined by finding the allocation site for each object, which is within its allocator's field data. Walking the chain of the allocator's predecessors and successors, a concrete network is established for all the associated objects that reside in the process image. The evaluation of the proposed approach on real applications shows the reconstruction and visualization of the object allocation network can aid investigators in finding the context for forensically interesting data such as deleted Whatsapp messages and malware data leaks.

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### Memory Forensics, Android™, Visualization

## C4 Performing Mac® Memory Analysis Using Objective-C and Swift Data Structures

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**Learning Overview:** After attending this presentation, attendees will have learned to write plugins for popular memory forensics frameworks after understanding how these plugins actually work. Attendees will become acquainted with Objective-C and Swift runtime data structures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method to find information about the source code of any Mac® application written in Objective-C or Swift from a memory snapshot. This method will reveal the classes, methods, and instance variables in the source code of a running application even though the actual source code or executable is not available to disassemble.

Memory analysis, also known as memory forensics, is the process of examination of the physical (volatile) memory of a computer system. Memory forensics has become very popular as it allows the recovery of a wide variety of artifacts that are not written to the hard disk and therefore unavailable when performing disk forensics. However, accurate forensic information can only be found if the forensic investigators have access to proper memory forensics tools. After attending this presentation, attendees will: (1) be acquainted with the state-of-the-art memory forensic techniques provided by popular frameworks such as Volatility; (2) understand how the plugins provided by these frameworks are written; and (3) apply the knowledge to design their own memory forensic tools. Furthermore, this presentation will provide a detailed discussion on the source code of the Swift and Objective-C runtime data structures. The attendees will therefore have a chance to understand the memory layout of the Objective-C runtime and how Mac® applications or malware written in Objective-C/Swift behave “under the hood.”

One of the main challenges in computer security is that the source code or the executable of an application or malware may not be available. In such a scenario, where a copy of the executable is not available and therefore disassembling to reverse engineer the source code is not an option, performing memory analysis can help recover information about the classes, methods, instance variables, and values of instance variables used. Memory analysis can be performed on a memory image of the device on which the required application is actively running. This presentation will discuss how the aforementioned technique would work for Mac® applications, how these applications are stored in memory, and how the Objective-C runtime data structure layout can help find information about the application source code.

Today, macOS® is considered one of the most popular operating systems and Objective-C and Swift are the “official” languages of Apple®. Most Mac® applications (and much of the malware) are therefore written in Objective-C and Swift. Apple® has made Objective-C and Swift runtime code open source.<sup>1,2</sup> The Objective-C runtime source code can be used to determine the memory layout of the runtime data structures. This information is then used to write new Volatility plugins. Volatility is one of the most popular memory forensics frameworks, regularly used in forensic investigations.<sup>3</sup> The Volatility plugins are written in Python®.

The results of this research enumerate the names of the classes, methods, and instances used in the source code of a given Mac® application or malware. The objects and meta-classes (containing metadata of classes) are used to find the values of the instance variables.

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### Memory Forensics, Mac® Operating System, Objective-C



## C5 AI-Based Audio Enhancement May Cause False Evidence

*Eddy B. Brixen, BA\*, EBB-Consult, Smorum DK-2765, DENMARK*

**Learning Overview:** The goal of this presentation is to raise awareness to the new pitfalls in the art of voice enhancement. This presentation underlines the necessity of personnel's scientific education to avoid false evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how Artificial Intelligence (AI) offers far more advanced possibilities regarding audio enhancement and how to avoid false conclusions.

**Introduction:** It is a common task to enhance low-quality speech recordings of various origins. Recordings from analog or digital wiretaps, surveillance systems, dispatch logging, dictaphones, memo recorders, etc. may end up at the audio forensics specialist's lab. Based on knowledge, training, and available equipment/software, the specialist may choose and apply different audio tools, primarily various filters, to enhance the recordings. Formerly, it was adequate to implement fixed filters or adaptive filters to reduce the recordings' background noise. Now AI offers far more advanced possibilities. Some algorithms are designed to take care of the noise in quite intelligent ways. However, other newer and more advanced algorithms look into signals recognized as speech. This is excellent—if speech is present!

Recently, a new type of recording has been introduced to audio forensics experts. These are recordings in which whispering or fragments of words seem to be recognized. At least, this is what the client states. Private clients and even clients from law enforcement may have the opportunity to acquire software that is extremely easy to use—or rather, from which it is easy to obtain a result. However, without the right training and the basic understanding of what the software actually does, these people may misinterpret the results.

AI-based dialog-isolating algorithms basically look for any voice content and emphasize this part of the signal. As an audio forensic expert, one should always detect whether or not recordings underwent any enhancement before arriving for analysis. Comments from the client like: "This speech was not audible until the filtering was performed" should always make one suspicious. The first time it is heard, there may be doubt as to whether or not speech is present, which one should not have.

**The Experiment:** After a number of challenging cases, this study carried out a series of experiments to identify the problem. At first, audio files were created by recording low-level speech close to the bottom of the dynamic range, applying linear recording as well as various versions of bit compression implemented in mobile devices. The speech was masked partly by quantization noise, partly by bitreduction artifacts, and partly by background noise (room noise). The files were enhanced applying Dialogue Isolate, a module of the widely used software iZotope RX8. The degree of processing was determined by the software's "Repair assistant," a tool suggesting the amount of treatment. The files were assessed by listeners, and by analyzing the Long-Time Average Spectra (LTAS), to find the degree of signal "voice-shaping."

The result of this test showed that syllables of spoken words—especially from cell phone recordings—may change to an unacceptable degree. Further, the process may generate unidentifiable speech-like sounds. It was not possible to identify a clear "voice shaping" profile of the signal.

After this part of the experiment, it became obvious to take it one step further. In this step, "voice enhancement" was applied to files not containing any speech. Different noises (pink noise, babble noise, low-level bit-reduction artifacts) were recorded using both linear quantization and bit compression. The recordings were assessed by listening. The result of this experiment exhibited a surprising effect: No recognizable words, of course. However, noise that fell within the frequency range of speech was emphasized, leaving speech-like sounds on the recording.

In cases where the question would be whether or not persons were present in a room, the described enhancement process could be hazardous to follow as it might lead to wrong conclusions.

**Conclusion:** This presentation describes new pitfalls in the art of voice enhancement. It underlines the necessity of personnel's scientific education to avoid false evidence. This presentation is supported by relevant sound samples from the experiment.

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### Audio Enhancement, AI (Artificial Intelligence), False Evidence

## C6 Connected Objects (Internet of Things [IoT]) as Crime Witnesses

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**Learning Overview:** After attending this presentation, attendees will be more familiar with traces from IoT devices in smart homes and understand how they can be used in an investigation context. The objectives of this presentation are to: (1) become more familiar with the different sources of traces available from IoT devices, such as traces on the smart phone, on the network, and on the cloud (available through personal data access); (2) demonstrate the investigative and forensic value of the traces in smart homes; and (3) illustrate how traces found during a simulated fire investigation helped with the reconstruction of the event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the different sources of traces from IoT devices in smart homes and the value they have during an investigation. This presentation will also demonstrate how digital traces can be used in any case, such as fire investigation, to reconstruct real-world events.

Connected objects have invaded our daily lives. They are therefore more and more present in investigations of all kinds. Connected devices are witnesses that record and store data related to its environment. Since the connected devices have little memory, the data generated by these objects is stored elsewhere, remotely on the phones associated with them and in the cloud. Thus, traces generated during a fire, for example, are not necessarily destroyed when the connected device is destroyed or damaged.

A smart home was created for this study, and different scenarios were run. It was found that traces could be present on the network, on paired devices such as smart phones, and in the cloud. The scenarios reproduce, first, a normal activity and, secondly, activities taking place during a fire. Thus, it was necessary to study the behavior of the devices in case of electrical short circuit, network failure, and submission to heat.

Concerning the exploitation of network captures, it was possible to observe, on the one hand, with whom the objects communicated, and, on the other hand, the normal traffic generated by them. It was also possible to determine causes of disruption (electrical, network, or heat) using the presence or absence of traces, and when it happened. This information can guide the search and analysis of physical traces.

Traces from the cloud could be seen as latent traces in this context; traces are unknown to the investigator and have to be “revealed.” Thus, this data is obtained using requests for personal data access (i.e., General Data Protection Regulation [GDPR] requests). It requires access to the user account but is quick and easy to obtain. The amount of data available depends on the device. It allows information on the user’s interactions with connected devices and the environment to be obtained. Most of the available data is also time-stamped, which is useful for establishing a chronology.

In conclusion, systematically identifying the objects present on the scene and getting as many credentials about them as possible is recommended. In addition, it is necessary request access to personal data as soon as possible. This precious information will help in reconstructing digital and physical activities.

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**IoT, Network Analysis, Personal Data Access**

## C7 Enhancing Child Pornography Offender Risk Assessment Using Digital Forensics Artifacts

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**Learning Overview:** After attending this presentation, attendees will: (1) understand the current research on the heterogeneity of Child Porn Offenders (CPO); (2) be able to describe the limitations of the current sex offender risk assessments and CPO; and (3) be able to discuss how using digital forensics artifacts can improve the risk assessments of CPO.

**Impact on the Forensic Science Community:** Our legal justice system is literally swamped with online child pornography cases. The current approach of treating all child sex offenders as one homogenous group for the purposes of risk assessments is overburdening the system and these assessments have low predictive validity for CPO. This presentation will impact the forensic science community by explaining how the new model will help improve the predictive validity of the risk assessments and help reduce the risk of contact victims.

While the legal justice system in the United States appears to be acutely focused on the prosecution of child pornography offenses, commonly referred to as Child Sexual Exploitative Materials (CSEM) offenses, there is surprisingly little empirical support for the classification of these types of sex offenders, the penalties, treatment, rehabilitation plans, and the use of risk assessment process/tools. The United States justice system seems to rely on anecdotal evidence at best, or at the very least, flawed stereotypes of who they believe these child pornography offenders are.<sup>1-3</sup> The goal of the legal justice system is not just to prosecute those individuals who are guilty of criminal offenses, it is also to protect those that have been victimized and hopefully prevent others from ever becoming a victim. If there is any hope of protecting against further victimization of one of our most vulnerable sectors of society, our children, then personal feelings, biases, and opinions must be put aside, and we must start understanding who these child pornography offenders are based on empirical evidence.<sup>4-6</sup> What is needed are long-term studies that look at the ontology of child pornography offenders, personality and demographic characteristics of potential categories of these offenders, and the real risk that these different categories of offenders pose to society.

The focus of this presentation is to provide a model that can be used to better understand the risk that CPO pose by combining traditional child sexual offender risk assessment tools and risk assessment tools designed specifically for CPO with digital forensic artifacts. This presentation will first look at the current research that indicates that the individuals that engage in child sexual exploitative behaviors are not a homogeneous group.<sup>3,4,7,8</sup> This presentation will examine how the current offender risk assessment tools used with traditional child sexual offenders do not seem to be up to the task and have poor predictive validity for CPO. Rather than trying to abandon and/or create new risk assessment tools for this population of offenders, which to date has not been very successful, it makes more sense to look at supplementing these assessment tools with data/evidence derived from digital forensics analyses.<sup>3,9,10</sup> Thus, a model is provided for supplementing the risk assessment tools. This model combines digital forensic artifacts, evidence visualization, current risk assessment tools, and a better understanding of the factors that are correlated with an increased risk of CPO reoffending or progressing to more serious criminal behaviors such as contact offending.

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### Digital, Risk, Porn

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## C8 New in Computer Forensics Tool Testing's (CFTT's) Mobile Forensics—SQLite, SQLite Recovery, and a New Federated Testing Tool

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**Learning Overview:** After attending this presentation, attendees will be familiar with the three major updates CFTT is working on to enhance its testing methodology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the current updates happening within CFTT's Mobile Forensic project and how the updates will be beneficial to forensic tool users in general.

The SQLite extension to the mobile forensic tool testing and the new specification for SQLite data recovery will provide a more thorough set of results to the forensic community. There are forensic tools designed or focused on SQLite data recovery, thus the creation of a new and separate specification tailored to SQLite data recovery only. This will inform the users about the handling of SQLite database files by the tools used during an investigation.

In addition, CFTT is working on a new Federated Testing tool. There are multiple forensic tools on the market with different versions, as it is expected. The Federated Testing project is an expansion of the CFTT program that provides test suites, using National Institute of Standards and Technology (NIST) methodology, for tool testing within the labs. The test suites allow users to test their own tool version, especially if it has not been tested by the CFTT project. The new version of Federated Testing will be portable, allowing users to run it on Windows® 10 without the need for installing any software.

The goal of the new project, within CFTT, and expansions of existing specifications is to establish and enhance the testing methodology to then apply it to newer forensic tool versions. This will provide tool makers with necessary information to improve their tools, users with would gather more information that will help them select the appropriate tool(s), and, last, help interested parties to understand the tool's capabilities.

*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, SQLite Data Recovery, Federated Testing



## C9 An Initial Forensic Analysis of Sailfish OS

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**Learning Overview:** The goal of this presentation is to address a gap in the forensics analysis of Sailfish OS. This presentation focuses on mapping the digital artifacts pertinent to an investigation, which can be found on the file system of a phone running Sailfish OS 3.2. Currently, there is no other known publicly available research and very few commercially available solutions for the acquisition and analysis of this platform. This is a significant gap, as this Operating System's (OS's) adoption is accelerating in emerging markets on low-cost devices in countries like Russia, China, and India. This presentation documents many of the significant forensics' points of interest, such as call and text, log, phonebook, web browser artifacts, and hardware-specific features.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing extensive detail on analyzing mobile phones running Sailfish OS. Sailfish OS is a Linux® kernel-based, embedded-device operation system, mostly deployed on cell phones. Currently, there is no sufficient research in this space. Simultaneously, this operating system is gaining popularity, so it is likely investigators will encounter it in the field.

This system has been rapidly deployed in Russia, India, and China. A clear example of rapid deployment is the mass deployment of eight million hand-held devices planned by the Russian government by the end of 2021. In India, the OS is deployed by some of the major network providers, and in China, Huawei is investigating it as an Android™ replacement, as economic tensions with the United States arise.

This presentation shows the mapping of the digital artifacts, pertinent to an investigation, which can be found on the file system of a phone running Sailfish OS 3.2. It covers call logs, text messages, location services, address books, and other important artifacts. The analysis was conducted based on a comparison of the image of the phone before and after controlled changes were introduced.

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### Sailfish OS, Cellphone, Artifact Analysis

## C10 An Analysis of Audio Recordings Made Using the Voice Recorder Application on Android™ Phones

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**Learning Overview:** The goal of this presentation is to explore the user interfaces for four different Android™ phones (HTC ONE®, Samsung™ Galaxy™ S6, S9, and LG® Q6) with four different voice recording application versions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining that the abilities to properly identify, examine, and verify information from a mobile phone are very important aspects of forensic research. Different applications and their capabilities are also important to research and understand, not just for the scientific community, but also for individuals who use these devices every day. This includes voice recorder applications that come standard on Android™ phones.

**Hypothesis:** The characteristics of each voice recording version on different Android™ mobile phones can determine whether the recording is authentic with that device and whether the recordings have been changed/edited.

Android™ is one of the two main operating systems for mobile phones and includes an application for voice recording. Since extensive research has been completed on the “Voice Memos” application for iOS®, there remains research to be completed on the default application for the Android™ OS. This presentation explores the user interface for four different Android™ phones: HTC ONE®, Samsung™ Galaxy™ S6 and S9, and LG® Q6, with four different voice recording application versions. Features of the recording application and its limitations as well as editing capabilities, file formats, and atom structure for each phone are discussed. All devices tested allow the ability to share the recordings via messages, email, Android™ Beam, Bluetooth®, and Gmail®. The Samsung™ phones also allow sharing via Outlook®, OneNote®, Samsung™ Cloud Drive, and many others, depending on what other communication apps are installed on the phone. All devices, except for the LG® Q6, allow the files to be renamed, deleted, shared, and a few other options. All devices, except for the HTC ONE®, allow for some type of editing. Testing protocols were created and utilized for the creation of the test recordings. These testing protocols were further established for the LG® and Samsung™ phones since their voice recording apps offer the most features. These recordings included continuous and paused recordings, interrupted recordings, and various ways of saving and storing the recordings.

Each phone with its different Android™ versions and different app versions offered different results based upon whether the recording was edited or unedited, continuous or paused, all of which contain important information when it comes to the analysis of these recordings and the authenticity of these recordings as related to each phone. Given that each phone comprises different user interfaces and different results, it is important for investigators to be aware of the features and limitations for each app. The consistencies between edited and unedited recordings also supply examiners with much-needed information. There are many other aspects of the Voice Recorder app to research, but this presentation provides a plethora of information that was not previously available.

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**Android™, Audio, Voice Recorder**

## C11 A Forensic Analysis of Digital Speech Standard (DSS) Files

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**Learning Overview:** After attending this presentation, attendees will better understand the unique properties that the DSS and DSS Pro (DS2) file formats contain in order to better assist with authentication of such recordings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing detailed authentication information for forensic analysts to use while following best practices for authentication of recordings purported to be from these types of recorders.

This work presents an extensive study on DSS and DS2 files created with Olympus® and Philips digital audio recorders. The authenticity of digital audio recordings can be challenged or should be verified either before being accepted as evidence in legal proceedings or in ascertaining the veracity of an unknown file's meaning.<sup>1</sup> The framework for forensic authentication of digital audio includes the structure and format analysis that requires the investigation of the suspected recording device as well.<sup>2</sup> Sometimes the suspected recorder is not available for test reference recordings, which could lead some labs to reject the request for analysis. One of the goals of this study is to provide the scientific community with a framework to better help authenticate known and unknown recordings from the listed recorder types/models.

Over 1,000 sample recordings were taken from multiple models of both Olympus® and Philips digital audio recorders. Careful consideration for the various recording quality settings was used, ensuring that sufficient amounts of recordings were made at each level of quality available with each recorder. These are usually listed as Standard Play (SP), Long Play (LP), or Quality Play (QP) quality in the devices. Many settings remained consistent across the brands/models, but it was made sure that samples were collected from any setting that resulted in a DSS or DS2 file format. The only settings not used were those that resulted in something other than a DSS or DS2 file format, such as MP3, WMA, PCM-WAV, etc. A large subset of these recorders was also tested with both internal and external microphones to determine if the recorder left any traces of recording source in the metadata. The models tested include: (1) Olympus® DSS: DM-1, DM-10, DS-2, DS-20, DS-330, DS-2200; (2) Philips DSS: DPM-6000, DPM-930, DPM-9450, DPM-9600; and (3) Philips DS2: DPM-6000, DPM-9600.

This presentation continues previous research on WAV, MP3, WMA, and AAC files.<sup>3</sup> It presents the principles followed to collect and analyze reference samples, reports the findings, and proposes a methodology to analyze DSS and DS2 file structure and format for forensic purposes.

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### Audio Authentication, Metadata, Digital Audio Recorders

## C12 Data Decryption of Android™ Third-Party Private Messaging Apps: A Case Study

Lyndsay Haak, BS\*, Marshall University Forensic Science Center, Huntington, WV 25701; Josh Brunty, MS\*, Marshall University, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will understand how the app CoverMe operates and how messages sent through the app are decrypted. This presentation will introduce the basics of CoverMe, the challenges with decrypting messages sent in this app and the measures taken to decrypt these messages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how to decrypt messages sent in third-party private messaging apps, specifically CoverMe, to help investigators expand their investigations and develop new techniques in their investigative process.

The increasing number of third-party private messaging apps has allowed users to send messages through the use of encryption without the worry of someone else being able to see the messages they are sending. One private messaging app in particular claiming to have “military-grade encryption” is CoverMe. CoverMe allows users to send messages at different security levels, along with the ability to remotely delete, recall messages, and set messages to self-destruct. The app also offers many other features such as private calling, private phone numbers, and a private vault. Users also have the option to set up decoy passwords along with disguising the CoverMe app to appear as a news reader app. An additional security feature of the app is that with each login attempt, it takes a picture to capture who is logging in and alerts the owner of the account.<sup>1-3</sup>

The security of this application and others like it may lure users to use these applications to conduct criminal activity due to these apps claiming their encryption to be “crack-proof” and very difficult for law enforcement and others to recover the artifacts relating to the investigation.<sup>3</sup>

A recent case presented to the laboratory presented a Samsung™ Galaxy™ S8 with suspected messages related to the crime sent and received in the CoverMe app. When extractions were performed on the phone, they appeared to be unsuccessful in decrypting the CoverMe messages. A case study was performed to determine if there was a method that allowed CoverMe messages to be decrypted that would allow the messages on the evidence phone to be recovered.

Using two Samsung™ Galaxy™ tablets, test messages were created in the CoverMe app. A physical extraction and file system extraction were performed and analyzed to determine what the CoverMe messages looked like. The messages appeared to be encrypted, and the contents of the messages were unable to be determined. The tool was able to show the login attempts along with the captured login images. Following the extractions, a secondary tool was used to see if their statement of being able to decrypt CoverMe messages held true. The physical extraction and file system extraction were uploaded into the secondary tool. The file system extraction appeared to look similar in the secondary tool as it did in the original extraction; the messages still appeared encrypted. The physical extraction when uploaded to the secondary tool was able to decrypt the messages at all security levels along with identifying the user they were being sent to and if they were incoming or outgoing. CoverMe messages sent at various security levels can be decrypted, though a physical extraction is required due to it being able to obtain a bit-by-bit copy of the phone, including hidden or deleted content.

Knowing CoverMe messages can be decrypted, the behavior of the app was then studied to aid in determining the Application Programming Interfaces (APIs) the app is calling. Studying the calls allowed for the determination of where the encryption key was being pulled from. Obtaining the encryption key CoverMe uses aids in understanding how CoverMe and other third-party messaging apps are encrypting their data.

### Reference(s):

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### CoverMe, Decryption, Mobile Device Forensics



## C13 A Forensic Analysis of Joker-Enabled Android™ Malware Apps

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**Learning Overview:** This presentation will introduce the basics, challenges, and limitations of the current Android™ malware detection and provide a detailed explanation about the usefulness and availability of information about the list of permissions required and potential private information leakage by malicious apps (e.g. <http://wap.thaiza.com/> → browser cookie and timestamp) In addition, the presentation will elaborate our methodology and large-scale experimental evaluation of the approaches used to build android malware analysis databases. Overall, application developers and researchers will learn how to take advantage of the database and search the analysis result for certain android packages to prevent app code being infected by malware.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing detailed information about a well-known malware named Joker and its techniques of avoiding detection by a major vetting process.

This project aims at developing a set of automated Android™ malware vetting tools to discover all the malicious behaviors of Android™ malwares in the forms of files in the local storage, SQLite database, or data sent to remote third-party server(s), to establish a dictionary-like Android™ malware database that includes malware themselves (malicious code and variant) with all the detected Internet Provider (IP) addresses, Uniform Resource Locators (URLs) and malicious behaviors as well as other types of evidence data (e.g., the list of permissions required).

In addition, this presentation will demonstrate a well-known malware named Joker (also known as Bread), which has infected over 17,000 Android™ apps since its first release and has evolved into numerous different variants. Both static and dynamic program analysis approaches (Evihunter) were applied on analyzing the malicious code, detecting malicious behaviors, and retrieving evidentiary data like the file path and its corresponding evidence types. Through the analysis of code, it was discovered that Joker not only leverages all kinds of cloaking and obfuscation techniques in an attempt to be undetected, but also uses dynamic package loading to hide its malicious payload. In order to automate the fraud subscribe process, Joker developers utilize injected clicks, custom HTML parsers and Short Message Service (SMS) receivers so that it will not require any interaction from the user. When the infected app gets installed, it carries out either SMS fraud, which sends text messages to premium-rate numbers or Wireless Application Protocol (WAP) billing fraud where a user's mobile account will be paying for the charges of the subscriber's bill. According to Google's® recent report, having three or more active variants of Joker on their official app market at the same time is very common and at peak times of activity, there are up to 23 different versions of Joker family submitting to the Google® Play Store in one day.

As many different variants are active on the air, 12 samples were collected from 46 infected apps that have been removed from Google® Play Store. In some versions of the Joker variants, the final payload is delivered through a direct URL obtained from the listed Command and Control (C&C) server. In these variants, the C&C address has been hidden in the code utilizing the string obfuscation where the string "sticker" was used to break the C&C address and hide it from the simple grep or string search in order to pass the vetting process. In some versions, the infected Google® Play app uses a stager payload to retrieve the final payload where the stager payload URL was encoded in the code and was encrypted using Advanced Encryption Standard (AES). Once an infected app gets started, it downloads the stager payload first, then utilizes the stager payload to execute the malicious final payload. In addition, it was discovered that some variants of Joker even leverage two-stager payload downloads in order to retrieve the final payload. As for these infected apps, they download the stage one payload, which downloads the stage two payload, which finally loads the malicious Joker payload.

### Mobile Forensics, Android™ Malware, Digital Evidence

## C14 Examining the Impact of Garbage Collection and Process States in Userland Memory Forensics

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**Learning Overview:** After attending this presentation, attendees will understand the effect of some critical external factors of the mobile runtime environment that impact userland memory forensics. The research primarily aims to highlight the impact of Garbage Collection (GC) and process states on the reliability of memory analysis as a crucial and important forensics technique.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the effect of critical runtime activities in the acquisition of memory and recovery of important evidence.

An efficient memory dump acquisition technique is required for useful memory analysis. Android™ GC can impact the availability of evidence as it is an automatic process that removes unused objects from memory. This research will demonstrate how GC and process states can affect data recovery by identifying different combinations of GC and process states introduced by Android™ Runtime (ART). The GC states include GC triggered and GC not triggered. Android's™ different process states are foreground, background, visible, service, and empty.<sup>1</sup> This research predominantly focuses on the foreground and background process states. First, the foreground state is the state when the user interacts with the application. Then, the background process state is when the application under execution is not visible to the user, but the runtime maintains the application in a dynamic list containing processes that are in execution.<sup>1</sup> The various combinations of GC and process states include: foreground and no GC triggered; foreground and GC triggered; background and no GC triggered; and background and GC triggered. This research acquired the real-time application dump for each combination and identify differences in data recovery.

In newer Android™ versions, ART allocates objects using RegionSpace and LargeObjectSpace allocators that trigger the GC for efficient memory utilization. The RegionSpace allocator allocates small objects like primitive objects, and the LargeObjectSpace allocator allocates large objects like multimedia in memory.<sup>2,3</sup> If the application creates many objects, then the Android™ Runtime environment triggers GC frequently.<sup>2</sup> As a result, the number of objects allocated and deleted during the runtime is unknown to a forensic analyst or the user. Thus the impact of GC and the process state changes triggered by the runtime is the primary focus of this research.

Acquired memory dumps were evaluated with tools like DroidScraper and VCR in recovering objects from RegionSpace and LargeObjectSpace.<sup>2,3</sup> In this work, the memory dumps acquired from the same application in different combinations of GC and process states are analyzed in depth with the tools mentioned above to monitor the number of objects retrieved. The results demonstrated differences in objects retrieved for certain combinations; for example, more objects retrieved when GC did not trigger, and process state was the background. Hence, a hypothesis opens a new research dimension to explain which objects get removed and when they are removed to illustrate the effect of external factors on the reliability and accuracy of memory forensic tools on a multi-app platform such as Android™.

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- <sup>3</sup> Saltaformaggio, B., Bhatia, R., Gu, Z., Zhang, X., and Xu, D. (2015, October). VCR: App-agnostic recovery of photographic evidence from Android device memory images. In: *Proceedings of the 22<sup>nd</sup> ACM SIGSAC Conference on Computer and Communications Security* (pp. 146-157).

### Garbage Collection, Process State, Userland Memory Forensics

## C15 An Analysis of Acquisition Methods of Ring Video Doorbell Files

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the different methods available to acquire Ring Inc. home security video files as well as the different quality and attributes available with each method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying the available information to examiners (video resolution/quality, availability of metadata, embedded timestamp) when acquiring Ring video files through different methods, resulting in a more informed approach to file acquisition.

Ring Inc. has millions of users and has partnered with over 500 law enforcement agencies nationwide in providing access to video that is streamed to the Ring Inc. servers.<sup>1</sup> Should that video capture any criminal or other activity that the user wishes to share, there are at least eight ways that the user could transmit the video using methods provided within the Ring Inc. website and mobile app. When applicable, video files can also be obtained directly from Ring Inc. through legal process or by law enforcement through the Ring Neighbors Public Safety Service (NPSS). An additional method of acquisition is also available using common website debugging tools.

Current best practices suggest legal service directly to the service provider to obtain video files as the service provider would likely provide the best quality video and contain the most available data for examiners.<sup>2</sup> This study is not aware of any research that has been conducted verifying this guidance and/or evaluation of video files received from Ring video doorbells compared to other acquisition methods. Additionally, Ring Inc. provides no documentation and/or guidance as to the best method to download/acquire video files. Legal service to the provider is often more time consuming and puts an additional burden on the investigator/examiner, the judiciary, and the provider, yet may not yield any better results.

Being that there are at least eight different methods of acquisition/download, it is possible that different methods may yield different results (e.g., resolution, metadata, embedded timestamp). In order to fully evaluate these variables, research was conducted on Ring video doorbell files acquired/downloaded using eight different methods. File format and structure was evaluated to identify methods that contained the same data as well as methods that could produce additional information that could benefit examiners (i.e., embedded timestamp, file creation metadata).

The results of this research can serve to help examiners make informed decisions on the best method of acquisition based upon specific examination needs. This research could also assist examiners/investigators in identifying the provenance of purported Ring video doorbell video files submitted to them. This information could not only help authenticate videos but ensure that the best available video files are used in any subsequent examination.

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### Video Forensics, Cloud Data, Video Acquisition

## C16 A Study on Unmanned Aircraft Systems Forensics Framework (UAS FFWK)

Nagi Mei, DSc\*, Arnold, MD 21012

**Learning Overview:** After attending this presentation, attendees will recognize the need for a standard UAS FFWK across digital forensic disciplines and aircraft accident investigation for use by investigators to produce admissible evidence for litigation in the United States courts. This presentation is based on research to identify common criteria in UAS forensics, across digital forensics and aircraft accident investigation guidelines, to determine if a single framework is needed for UAS forensic examiners.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to diligent forensic investigations in the justice system. This presentation will impact the UAS forensic community by relaying the results of research conducted on the use of the UAS forensics framework.

Few studies exist on the need for a standard UAS framework, and FAA UAS Regulations and Policies (2017a) are relatively new for consumer drones.<sup>1</sup> As consumer UAS or drones are used more commonly in public areas, an approach for a UAS FFWK is needed to guide forensics investigators in analyzing recovered drones that entered the unauthorized National Airspace System (NAS). With the increased use of small Unmanned Aerial Vehicles (UAVs) in public areas, a framework is needed to analyze the recovered consumer drones that trespass into the NAS.<sup>2</sup> Increased drone presence in the common airspace will force the integration of UAS into the NAS, which poses a risk to public safety.<sup>3</sup>

This research study focused on whether a single framework and multiple frameworks for UAS forensics was needed. The data collected through an online survey provided detailed descriptions of the participants' most commonly used forensics frameworks or guidelines. The data collected from participants in the United States measured the occurrences of the type of guidelines forensics examiners used. The study identified and evaluated existing frameworks, tools, and regulations investigators used to perform UAS forensics. The study results identified a need for a standard UAS forensic framework across digital forensic disciplines and aircraft accident investigation for use by investigators to produce admissible evidence for litigation in the United States courts. This research led to a proposed basic UAS FFWK, which can be improved upon in future research by building onto the existing aircraft accident investigation and digital forensics models reviewed in this presentation.<sup>4</sup> Future research should include detailed processes for each area of the proposed UAS FFWK. The UAS FFWK may also apply to other unmanned vehicles on land or sea by adapting the general framework.

### Reference(s):

1. FAA. *Law Enforcement Guidance for Suspected Unauthorized UAS Operations*. Published online 2018:1-12. Accessed October 11, 2020. [https://www.faa.gov/uas/public\\_safety\\_gov/media/FAA\\_UAS-PO\\_LEA\\_Guidance.pdf](https://www.faa.gov/uas/public_safety_gov/media/FAA_UAS-PO_LEA_Guidance.pdf).
2. Jain U., Rogers M., Matson E.T. Drone forensic framework: Sensor and data identification and verification. *SAS 2017 - 2017 IEEE Sensors Appl Symp Proc*. Published online 2017. doi:10.1109/SAS.2017.7894059.
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### UAS Forensics Framework, Drones, Unmanned Aircraft

## C17 A Response to the Threat of Stegware

*Li Lin, Iowa State University - Mathematics, Ames, IA 50010; Abby Martin, BA\*, Iowa State University, Ankeny, IA 50023; Wenhao Chen, BS, Iowa State University - Coover Hall, Ames, IA 50011; Seth H. Pierre, Ames, IA 50014; Yong Guan, PhD, Iowa State University, Ames, IA 50011; Jennifer Newman, PhD\*, Iowa State University, Mathematics Department, Ames, IA 50011*

**Learning Overview:** The goal of this presentation is to give a survey on the performance of the most popular steganalysis tools that claim they can detect the images with secretly embedded information by stegware. Recent research papers and algorithms for the defense against stegware that may have a chance to stop the threaten from stegware before the installation will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the current situation of steganography software (or stegware for short) and steganalysis tools for illegal purposes, such as how popular and efficient they are. This will be the first report to talk about the performance of the most recent steganalysis tools in detecting a large set of stegware. The results will provide valuable guidance to the forensic communities to develop more powerful steg analyzers.

Stegware refers to software, programs, or apps that allow insertion of malware into a digital file, such as an image or video, using steganography techniques. Although it has been in action for around 15 years, “steganography” and “stegware” have just recently attracted the attention of law enforcement agencies as the use of stegware appears to be rising.<sup>1</sup> This technique has been used for international economic espionage, tracking of photos shared by users on social media platforms, and industrial and governmental espionage by hacker groups using Portable Network Graphics (PNG) images to hide malicious code.<sup>2-4</sup>

The war between the stegware and steganalysis tools is a typical cat-and-mouse game. Although many up-to-date steganalysis tools claim their abilities to prevent steganography by utilizing the most advanced detection algorithms from the academic worlds, these tools focus mainly on one or two embedding algorithms and lack support to detect a wide range of stego objects.<sup>5</sup> The capability of these current tools to prevent a stegware attack has never been tested.

In this research, more than 70 stego apps and image steganography software and ten of the most popular steganalysis tools were collected. A strategy is proposed to defend real-world attacks from stegware by combining functions from online steganalysis tools and algorithms from recent academic discoveries. This is believed to significantly increase the chance of identifying the threat from stegware by identifying files that have the potential to contain malicious code. Work is occurring to develop a prototype of such a comprehensive steganalysis tool that provides user-friendly software for non-experts such as digital evidence practitioners. The characteristics of the code for many stego apps are summarized by reverse engineering and program analysis. The coding characteristics reflect their core embedding algorithms and encryption techniques, allowing the classification of the intent of the app as stegware even before installing it on a mobile phone. An automatic tool to analyze app code can detect most Android™ stego apps that implement common spatial domain and frequency domain embedding algorithms with more than 95% accuracy.

Per research, this is the first study to evaluate the performance of most recent steganalysis tools in detecting a large set of stegware. The results will provide valuable guidance to the forensic communities to develop more powerful steg analyzers.

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### Digital Image Forensics, Steganalysis, Android™ Apps



## **C18 The Use of 3D Injury Maps and the Effects of Different Visual Presentation Formats**

*Sophena S Wilson, MS\*, Birmingham, West Midlands B30 2YW, UNITED KINGDOM*

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**WITHDRAWN**



## C19 The Organization of Scientific Area Committees (OSAC) Digital/Multimedia Scientific Area Committee Standards Work—Part 1: Digital Evidence and Speaker Recognition

*Lam D. Nguyen, MS\*, Woodbridge, VA 22192; David Brian Marks, MS\*, Federal Bureau of Investigation, Quantico, VA 22135; Richard Vorder Bruegge, PhD, Federal Bureau of Investigation, Quantico, VA 22135*

**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 Speaker Recognition.

**Impact on the Forensic Science Community:** Standards are vital to ensuring the sound practice of forensic science across all disciplines. 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.

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.

OSAC forensic science disciplines are spread across seven major Scientific Area Committees (SACs). The SAC most relevant to the AAFS Digital Multimedia Sciences section is the Digital/Multimedia SAC (DMSAC). The DMSAC incorporates four subcommittees that address the following disciplines: Digital Evidence, Speaker Recognition, Video/Imaging Technology & Analysis, and Facial Identification. This presentation will focus on the work of the first two, while a companion presentation addresses the second two.

Each OSAC subcommittee maintains a “roadmap” of standards and other guidance documents for their discipline and identifies the individual standards and guidance documents considered to be of the highest priority for their work. 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 as approved standards. 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 several 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 OSAC or through an SDO. The SDOs with which the OSAC Digital Evidence and Speaker Recognition subcommittees are currently engaged include: American Society for Testing and Materials (ASTM), the Acoustical Society of America, and American National Standards Institute (ANSI). It is worth noting that with changes implemented in the fall of 2020, standards developed in OSAC that have been passed to an SDO are now published on the OSAC Registry as “Standards Under Consideration.” This further enhances the transparency of the standards development process by making these documents available for all to see outside of the SDO and OSAC process.

To facilitate the process by which standards are reviewed and validated through the OSAC, the Forensic Science Standards Board (FSSB) has established an ontology to define the categories and subcategories of standards that may be included in the roadmaps. The primary categories and subcategories of standards as defined in this ontology are as follows: Competency; Physical Evidence; Method Development & Method Validation; Evidence Enhancement, Restoration or Recovery; Examination & Analysis; Interpretation; Quality Assurance; Terminology; and Reporting Results & Testimony. 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 and guidance documents within the disciplines of digital evidence and speaker recognition with such topics as: Mobile Device Evidence Collection, Preservation, Handling and Acquisition; Requirements for Testing Tools Used in Digital and Multimedia Forensics; Archiving Digital and Multimedia Evidence; Internet of Things (IoT) Devices; Mobile Device Analysis; Audio Collection at a Temporary Location; Issues in Data Processing and Relevant Population Selection; Taxonomy of Mismatch Conditions for Forensic Speaker Recognition; and a Process Map of Recommended Practices in Forensic Speaker Recognition.

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### Digital Evidence, Speaker Recognition, Standards



## C20 The Organization of Scientific Area Committees (OSAC) Digital/Multimedia Scientific Area Committee Standards Work—Part 2: Video/Imaging Technology & Analysis (VITAL) and Facial Identification

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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 VITAL and Facial Identification.

**Impact on the Forensic Science Community:** Standards are vital to ensuring the sound practice of forensic science across all disciplines. 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 Organization of Scientific Area Committees (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.

OSAC forensic science disciplines are spread across seven major Scientific Area Committees (SACs) The SAC most relevant to the AAFS Digital Multimedia Sciences section is the Digital/Multimedia SAC (DMSAC). The DMSAC incorporates four subcommittees that address the following disciplines: Digital Evidence, Speaker Recognition, Video/Imaging Technology & Analysis, and Facial Identification. This presentation will focus on the work of the latter two, while a companion presentation addresses the former two.

Each OSAC subcommittee maintains a "roadmap" of standards and other guidance documents for their discipline and identifies the individual standards and guidance document considered to be of the highest priority for their work.

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 several 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 OSAC or through an SDO. The SDOs with which the OSAC Digital Evidence and Speaker Recognition subcommittees are currently engaged include American Society for Testing and Materials (ASTM), the Acoustical Society of America, and American National Standards Institute (ANSI).

To facilitate the process by which standards are reviewed and validated through the OSAC, the Forensic Science Standards Board (FSSB) has established an ontology to define the categories and subcategories of standards that may be included in the roadmaps. The primary categories and subcategories of standards as defined in this ontology are as follows: Competency; Physical Evidence; Method Development & Method Validation; Evidence Enhancement, Restoration or Recovery; Examination & Analysis; Interpretation; Quality Assurance; Terminology; and Reporting Results & Testimony. It is expected that individual standards might span more than one of these categories.

During this presentation, attendees will learn about various efforts to develop standards within the disciplines of video/imaging technology and analysis and facial identification with such topics as: Categories of Results/Opinions in Comparison Analyses; Developing Discipline Specific Methodology for Analysis, Comparison, Evaluation-Verification (ACE-V); Digital Video Retrieval and Analysis; Image Authentication; Forensic Photogrammetry; Crime Scene Photography; Physical Stability of Facial Features in Adults; Image Processing to Improve Facial Recognition Searches; and multiple categories of training. In addition, attendees will also hear about a document developed in response to the corona virus pandemic that provides guidance for the remote/offsite examination of digital and multimedia evidence.

### Video/Imaging Technology & Analysis, Facial Identification, Standards



## C21 An Analysis of Body-Worn Camera Photogrammetry Using Depth Mapping

Toby M. Terpstra\*, KinetiCorp LLC, Greenwood Village, CO 80111; William T. Neale, MArch, KinetiCorp LLC, Greenwood Village, CO 80111; Tomas Owens, BFA, KinetiCorp LLC, Greenwood Village, CO 80111; Eric King, BA, KinetiCorp LLC, Greenwood Village, CO 80111; Steven Beier, MS, KinetiCorp LLC, Greenwood Village, CO 80111; Tilo Voitell, Douglas County Sheriff's Office, Castle Rock, CO 80109

**Learning Overview:** After attending this presentation, attendees will be aware of both the issues of contemporary body-worn cameras as well as the accuracy with which 3D information can be obtained from within the video using camera-matching photogrammetry. This is done through specific research and is demonstrated through a case example.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of how photogrammetric analysis can benefit use-of-force cases. Body-worn cameras are capturing an increasing number of events with controversial implications. Often, the events are fast paced and the video can be both disorienting and misleading. A 3D video analysis can bring greater understanding to the events as they occurred.

The initial push to have a video record documenting a police officer's encounter with suspects began in the early 1980s with the advent of the Mothers Against Drunk Driving (MADD) Campaign.<sup>1</sup> The need to document and record traffic stops, specifically drunk driving instances, was under heavy influence by the MADD campaign.<sup>2</sup> Since then, dash-mounted cameras have become common place in patrol vehicles and have expanded to include Body-Worn Cameras (BWC). In 2007, *NBC News* and the *Associated Press* reported that "Britain straps video cameras to police helmets."<sup>3</sup> Momentum from Europe's use of BWCs continued, transitioning to the United States in 2014 when Washington, DC, New York City, and Los Angeles began pilot programs.<sup>4</sup>

The video and audio record of an event captured by BWCs, as well as cell phones by witnesses and surveillance footage, have led to the emergence of issues surrounding the use of force. However, BWC footage can be blurry, distorted, shaky, and difficult to view due to the constant movement of the body to which the camera is attached, the limited vantage point and field of view of the camera, and movement of the camera from contact with other objects and personnel. To better understand what is actually occurring in the video, techniques of photogrammetry and videogrammetry have been developed to determine the position, orientation, and movement of objects and people involved in an incident that is captured in video. This research examines the issues and accuracy of using existing photogrammetric and videogrammetric techniques with BWC footage. Additionally, this research evaluates the techniques used to correct the factors that can make BWC footage different than other types of video recordings such as low light levels, low resolution, the distance from other objects, lens distortion, motion blur, limited field of view, and obstructions from the arms and hands of the officers themselves.

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### Video Analysis, Photogrammetry, Body-Worn Cameras

## C22 Determining Range of Certainty in Photogrammetry and Videogrammetry

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**Learning Overview:** After attending this presentation, attendees will have been presented with a straightforward methodology for determining range of certainty in both single image photogrammetry solutions as well as multiple image photogrammetry solutions. This methodology has the added benefit of relating the range in specific measured units and resulting imagery to visually demonstrate the range. Research comparing known errors and determined ranges of certainty will be presented to validate the methodology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a meaningful way of determining and visually demonstrating range of certainty in camera-matching photogrammetry and videogrammetry.

Photogrammetry and videogrammetry are utilized in the forensic industries to accurately measure the timing, orientation, and position of objects, people, markings, or other visible entities in photographs and video footage.<sup>1-3</sup> This research presents a methodology for documenting and reporting the range of certainty in photogrammetric and videogrammetric techniques and demonstrates the accuracy of this methodology through analysis of four separate studies. The first study evaluates the methodology through analysis of video using body worn Camera (BWC) footage, and the second study evaluates the methodology through analysis of static camera photographs of a staged vehicle accident. Participants were provided with a camera-matched, photogrammetric solution based on a single photograph or video frame and were instructed to position and orient the 3D models of people and objects so that they were aligned to those visible in the media. These positions were compared to known object positions to determine the placement error for each object. After alignment, participants were instructed to determine a range of certainty for these positions by incrementally moving each object they had positioned toward and away from the camera and left and right of the camera path until the 3D model exceeded the extents of a reasonable match and the 3D model was no longer in alignment with the corresponding object in the media. The range of certainty determined for each object by the participants was then compared to the placement error to evaluate what percentage of error fell within the participants' range of certainty.

The accuracy of a photogrammetric solution has been shown to improve through use of multiple images recorded from different vantages.<sup>4-6</sup> To evaluate the effectiveness of this methodology on photogrammetric solutions with more than a single photograph or video frame, a third study was done using three video frames from BWC footage, and a fourth study was done using three still camera photographs. Participants were instructed to align 3D models so that they were consistent with all three camera matches for each study, then determine a range of certainty for each study based on complete photogrammetry solutions. To demonstrate the effectiveness of this methodology, the comparative results of these studies are reported graphically and numerically.

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### Photogrammetry, Range of Certainty, BWC Video Analysis



## C23 Computer Forensics Reference Data Sets (CFReDS v2.0) for Digital Evidence

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**Learning Overview:** After attending this presentation, attendees will better understand the improvements to the CFReDS version 2.0 for digital evidence website. These reference data sets provide the forensic community with documented sets of simulated digital evidence for examination. CFReDS v2.0 is based upon a taxonomy that provides users with the search functionality narrowing hits specific to a dataset of interest. The CFReDS project at the National Institute of Standards and Technology (NIST) will provide users with a portal of datasets produced by the Computer Forensic Tool Testing (CFTT) program and other organizations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview on the functionality of CFReDS v2.0 and the importance of documented sets of simulated digital evidence.

Documented sets of digital evidence allow forensic practitioners, researchers, the general public, and others with the ability to validate software tools, insight to forensic tools' capabilities and limitations as well as training on a specific forensic task. Numerous types of datasets exist within the CFReDS repository, for example, scenario, technology, archived-based data sets that provide users with the ability to gain insight to a digital forensic tools functionality and perform proficiency testing for various types of digital forensic tools.

The new and improved CFReDS leverages a dynamic and ever-evolving taxonomy that includes nodes (or "tags") that can be used by the users to quickly filter out relevant datasets that are usually very information-dense. Using the taxonomy as a building pillar of the application moving forward, CFReDS intends to become a full-fledged search engine in the world of digital forensics and will include test reports and tools, allowing a three-way data correlation and completing the forensics lifecycle.

The goal of CFReDS v2.0 is aimed at providing the forensic community with a centralized repository portal of digital datasets created by numerous worldwide organizations. This centralized portal will provide the forensic community with the ability to quickly find datasets of interest within a centralized website.

The presentation gives an overview of CFReDS v2.0 and the importance of a centralized portal sharing data sets from numerous organizations across the country.

*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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### CFReDS, Digital Data Sets, Digital Forensics

## C24 Using Rapid Differential Forensics Algorithm to Speed Transmission of Large Files Around the World

*Mark D. Guido, MS\*, The MITRE Corporation, Mclean, VA 22102; Rob H. Schmicker, BS, The MITRE Corporation, McLean, MD 22102; Brandon Adler, MS, The MITRE Corporation, Mclean, VA 22102; Tristan Fletcher, BS, The MITRE Corporation, Mclean, VA 22102*

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**Learning Overview:** The goal of this presentation is to describe an extension to the previously presented Rapid Differential Forensics Imaging algorithm to address a common issue affecting forensic laboratories and practitioners around the world: their ability to efficiently obtain forensic acquisitions and co-locate them with their computing power.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a reference architecture that both saves significant amounts of time and money comparatively to how major laboratories are performing the practice today and also reduces the time to analytical results.

In 2016, an automated differential forensic imaging technique and algorithm was introduced that uses baseline datasets and hash comparisons to limit the amount of data sent from a mobile device to an acquisition endpoint. It produced forensically validated bit-for-bit copies of device storage in significantly reduced amounts of time compared to commercial products.

As is common practice today, forensic laboratories centralize their images and forensic computing power to minimize the time it takes to process the image and perform forensic analysis. The processes used, surrounding case details, and employed forensic analysis techniques may be considered sensitive to an investigation, which yields a general reluctance to decentralize or use cloud computing resources. Major laboratories often opt to physically mail large forensic images to their computing destination, causing significant delays in returning actionable results to assist an investigator or try to prioritize transmitted data to send, essentially taking a guess as to where relevant data is located on the hard drive to prioritize those areas to send, causing similar problems that they were trying to avoid during the acquisition phase. The United States military may similarly attempt to relocate forensic images to a centralized environment to return actionable, timely results to soldiers that may need it. In some environments, the soldiers may only have a low bandwidth (potentially unidirectional) transmission medium (e.g., satellite transmission) available to them to relocate their acquired data.

By applying an automated differential forensic imaging algorithm, the amount of time needed to perform a transmission from a sender to an endpoint is reduced. It eliminates sending duplicate or common parts of image files, including seen-before operating system file parts and empty unallocated space, as well as removing random, at-rest encryption from empty portions of the transmitted disk image. For unidirectional communications, the addition of forward error correction provides assurances that when data is lost or mangled, we can be assured we can still reconstruct it using the additionally transmitted parity information.

Further, when implemented on a cloud infrastructure such as Amazon<sup>®</sup> Web Services (AWS), it significantly reduces the time and cost it takes to send large files from one region to another and closes the gap between acquisition and returning analytical results to investigators. Although very fast, this inter-region computing can be expensive if utilizing AWS-provided communication mechanisms. By utilizing an algorithm to only send the data required by the algorithm to the destination, a savings on resources leads to reductions in time and cost savings.

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### Differential, Rapid Transmission, Cloud

## C25 A Comparative Analysis of Mobile Forensic Tools: Reliability and Accuracy Related to iOS® 13 Notes App Forensic Evidence Recognition and Classification

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**Learning Overview:** After attending this presentation, attendees will be able to describe the limitations of current mobile forensics tools and understand the impact that changing data structures have on digital forensics.

**Impact on the Forensic Science Community:** Mobile forensics needs the ability to independently validate forensic tools. Most vendors do not publish error rates, accuracy, or reliability measures for their tools. As underlying data structures change when updates to Operating Systems (OSs) occur, it is vital that we be aware of errors this may introduce. This presentation will impact the forensic science community by providing a simple framework for testing certain aspects of mobile forensics tools.

Evidence in digital forensic investigations is largely acquired through tools that are responsible for the acquisition, interpretation, and presentation of data from digital devices. Thus, it is crucial that the data produced by these tools be accurate, valid, and reliable; the failure of this could lead to a false conviction or set an offender free. The *Daubert* considerations put further pressure on the necessity to measure or have the potential to measure reliability, accuracy, and error rates.<sup>1</sup> Moreover, it can risk the credibility and reputation of the investigator. Currently, the most commonly used tool testing platform is the National Institute of Standard's and Technology's Computer Forensics Tool Testing Program (NIST - CFTT), but according to some literature this program is limited and has proven insufficient in properly validating the required tools.<sup>2</sup> This lack of a standardized tool validation system was the motivation for performing a comparative study to analyze the accuracy and reliability of three popular forensic tools in their ability to adapt to the SQLite database changes in iOS® 13 devices. The overall organization of file backups in iOS® is in the form of directories which contain SQLite databases, plist files, Extensible Markup Language (XML) files, text files, and media files. In every major update of the iOS®, the way in which data (potential evidence) is stored in these files seems to change (i.e., location and structure), which poses a challenge for forensic tools that need to keep up with the changes.<sup>3</sup>

The focus of this presentation is to provide the results of a comparative analysis of the following tools: (1) MSAB XRY®, (2) UFED Cellebrite®, and (3) Magnet AXIOM® in their ability to read and categorize Notes stored in the Notes app in iPhones® running iOS® 13 (locally stored, not cloud based). The Notes app has three categories of notes: (1) Active Notes—These are the notes that are created and currently stored in the app; (2) Recently Deleted (Marked for Deletion)—These are notes that the user has deleted, which are moved to a separate folder called “Recently Deleted,” which permanently deletes the notes in it after 30 days; and (3) Deleted Notes—These are permanently deleted notes, which do not exist in the “Recently Deleted” folder anymore.

The tools will be measured using two criteria: Reliability (the tool's ability to read the SQLite database and identify all three categories of notes) and Accuracy (the tool's ability to correctly place each note in the respective category). The results of the testing as well as suggestions for improving the tools will be discussed. The tool vendors will be provided with the results prior to the presentation.

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2. Hughes, N., and Varol, C. (2020, March). The Critical Need for Tool Validation before Using Malware Scanners in Digital Forensics. In *ICCWS 2020 15<sup>th</sup> International Conference on Cyber Warfare and Security* (p. 228). Academic Conferences and publishing limited.
3. Shimmi, S.S., Dorai, G., Karabiyik, U., and Aggarwal, S. (2020, June). Analysis of iOS SQLite Schema Evolution for Updating Forensic Data Extraction Tools. In *2020 8<sup>th</sup> International Symposium on Digital Forensics and Security (ISDFS)* (pp. 1-7). IEEE.

### iOS®, Tools, SQLite

## C26 Looking Into Your Future: A Continuous Human Gait Prediction for the Near Future

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**Learning Overview:** After attending this presentation, attendees will better understand the advances in biometrics research and how it is possible to use everyday devices such as mobile phones to predict the future actions of people.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel method for activity prediction, behavior prediction, and identification using mobile sensors, which leads to security improvements in monitoring systems.

Motion sensor recordings from users' mobile phones can be used for several applications, including authentication, activity, and gesture recognition. One of the challenges of these applications is the availability of data and the ability to make decisions in a short amount of time. This availability is dependent on user movements, and one of the methods that can help faster decision-making in these systems is to make future data available earlier by predicting the future movements signal.

Deep neural networks have achieved notable results in predicting the future based on observing a history of available data. Among those, Long Short-Term Memory (LSTM) has been used to forecast speech, handwriting, and other time-series data. This research builds a prediction model based on an LSTM neural network that learns the user's phone movement patterns during gait activity and predicts the near-future gait movements. The proposed model generates a future signal without having information about the activity that is being performed. It is trained with data from several gait activities such as walking and biking and predicts when similar activities are performed. This prediction has multiple applications in security systems, such as continuous authentication. Earlier availability of data helps continuous authentication systems make faster decisions. As another application in forensic science, the ability to predict the future activity of people can be used to detect if the person is likely to commit a crime or malicious activity and take action to prevent it. In contrast, predicting future gait movement signals can be used to replicate user body motions using a limited amount of data in order to exploit a security system. On the other hand, it can also be used to prevent the attacks when the system learns to forecast and replicate the upcoming motions and unforeseen events better than a human replicator. In the case of a robot replicator (attacker), the winner is the system that is trained with more data, which is usually the genuine system.

The experimental results on a dataset of four different activities performed by nine different users show that the prediction model forecasts the near-future gait movement signal with a small Dynamic Time Warping (DTW) distance between the predicted and the real signal.

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### **Biometric Security, Gait Identification, Movement Prediction**

## C27 A Holistic Framework for Investigating Geospatial Data in Cyber Forensics

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**Learning Overview:** After attending this presentation, attendees will be able to identify several implicit types of geospatial data/metadata formats that can be stored and recovered on different digital devices; determine the best forensics and analytics practices when it comes to dealing with geospatial data; and distinguish major geospatial concepts and qualities that can be considered beneficial and critical when conducting any digital/cyber forensics investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing extended capabilities when investigating geospatial data considering new metadata and increasing the awareness on the importance of geospatial data analysis for investigators and first responders.

Geospatial forensics is still a relatively new trend that deals with the examination of geodata that is integrated into many digital devices (e.g., Internet of things (IoT), smart phones, wearable devices, drones, autonomous vehicles, and robotics). Geospatial data has proven to be of high importance to digital forensics investigations.<sup>1</sup> Recently, the National Institute of Standards and Technology (NIST) pointed out challenges related to the identification phase of digital cloud forensics, where geospatial data have been considered an important component that can assist in finding evidence.<sup>2</sup> However, geospatial data are not always identified directly with the latest digital forensic tools. These tools depend heavily on the use of Global Positioning System (GPS) coordinates preserved in Exchangeable Image File Format (EXIF) tags or artifacts. This poses many challenges to investigators when examining geospatial data/geolocation information, represented in different data formats/schema that are not considered necessarily explicit geospatial format (e.g., Internet Protocol (IP) addresses).

Although digital forensics tools have attempted to cope with the mentioned challenges to recover geospatial data for many years, not all geospatial data formats have been taken into consideration. Moreover, there continues to be a lack of research that combines the identification and investigation of all geospatial-related types of data into one holistic investigative framework that uses multiple geospatial data analytics techniques, including geospatial Open-Source Intelligence (OSINT) and geospatial analysis. Furthermore, it is important to demystify different methods that investigations can use to fully identify information related to geolocation from a device to aid in drawing more accurate conclusions.

This presentation will illustrate the importance of the proposed geospatial holistic investigative framework to identify various geospatial data types and suggest possible geospatial examination techniques. Moreover, as many investigations happen to have devices that store geospatial data, the proposed framework will aim to look at the issue from different perspectives (e.g., geographically, forensically, and technologically). Furthermore, the proposed work will highlight the importance of spatial thinking and spatial awareness to enhance digital forensic investigators' current capabilities. Finally, the technical experiment conducted in this work will serve as a proof of concept, which will help demonstrate the newly proposed holistic approach that defines and sets the ground of all related geospatial data into the different forensics fields.

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### Cyber Forensics, Geospatial Data, Geospatial Forensics



## **C28 Crowdsourcing Forensics: Generating a Digital Artifact Catalog**

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**WITHDRAWN**



## C29 File Structure Analysis of Media Files Transmitted and Received Over WhatsApp

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**Learning Overview:** After attending this presentation, attendees will be aware of changes to file structure and metadata found to be associated with images and audio files that have been transmitted and received over the WhatsApp social media application compared to their original counterparts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing analysts and investigators with information to help better understand the process of different uploading and downloading techniques on the WhatsApp application utilizing different devices and the effects these methods have on file structure and metadata.

WhatsApp is currently the third most popular social media network and the single most popular communication application worldwide with at least 1.6 billion active users. The application has garnered attention for its end-to-end encryption and the privacy it offers to users. There have been recently documented cases of criminal and terrorist organizations using WhatsApp to communicate and share files securely. The ubiquitous nature of WhatsApp in today's society, along with its use by those with nefarious criminal intentions, highlights the relevance and importance of the findings in this study.

Key identifying features of media file structures that can be attributed to files that are transmitted and received over WhatsApp from different types of devices will be analyzed. Law enforcement and other investigative agencies can use this information to help determine the source of image and audio files acquired during the course of forensic investigations.

This presentation proposes that as an image or audio file undergoes the process of being transmitted from one user to another over the WhatsApp application, that file is imparted with metadata and traces of compression unique to that process that can later be detected and identified. A dataset of hundreds of image and audio files is created by manually transmitting and then downloading a set of original images and audio files utilizing many possible transmission methods and devices. This set of files is compared to the original recorded files, as well as to each other, to identify unique file structure characteristics. Commonalities between all transmitted files is also discussed. File characteristics such as naming convention, metadata, quantization tables, and image and audio compression are examined.

Based on the results of analysis, three different image compression schemes were detected and identified as being applied to image files transmitted over WhatsApp. These compression schemes are shown to be applied to images depending on which of three broad categories of devices are used to send the image files. Analysis of hex data associated with the transmitted audio files was conducted, and a model of the audio compression applied by WhatsApp was detected and configured for a lossy audio compression database. The information presented here can be added to a growing volume of analysis conducted on media files transmitted and received over different social media platforms.

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### Digital, Multimedia, WhatsApp



## C30 A Technique For Reducing Flash Glare for the Forensic Photographer

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**Learning Overview:** The goal of this presentation is to highlight a photographic technique for capturing subtle detail by reducing glare within forensic applications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a photographic technique that can be employed to more accurately document difficult-to-capture details, often obscured by flash glare.

The Digital Single Lens Reflex (DSLR) camera and accompanied flash unit are tried-and-true equipment used during forensic science applications to document subject matter pertinent to a forensic investigation. Quite often investigators will strap on a digital camera and flash and click away, believing they are photographically documenting the scene or subject in the most true and accurate way possible. For many scenarios, this may be true, but in certain situations, the powerful, direct light from a flash will obscure subtle detail that may be relevant to a medicolegal death investigation by causing glare or hotspots on the resulting image.

Properly illuminating subtle findings on darker skin colors, particularly non-flat and spherical surfaces, can be especially difficult with a standard flash unit because of reflection causing glare and hotspots. Simply removing the flash unit from the camera and employing off-axis lighting will not suffice in fully capturing the subject as a whole. Examples of this that come up quite often are bruising and other defects of the head and very small particles (such as gunshot residue) deposited on curved surfaces of the body.

A technique used at the Harris County Institute of Forensic Sciences to accurately document subject matter where photographic flash may obscure the element of importance is to remove the flash unit, significantly increase the International Organization for Standardization (ISO) (sensor sensitivity to light), adjust the white balance appropriately, and use the digital camera's internal light meter for proper exposure. By increasing the ISO, the photographer is able to utilize ambient light to obtain the correct exposure without the aid of a tripod, saving precious time and avoiding precarious tripod positioning. A potential limitation of this technique is diminished resolution quality; depending on the ISO setting, increased pixel noise can result in a "softening" of the overall focus. This trade-off can be justified when used as needed, in conjunction with flash photography to illuminate the subject at hand. When removing the flash unit from the equation, the photographer is reliant upon ambient light to illuminate the subject, which may need to be corrected for in the white balance settings. While using only ambient light, the technique of transillumination (using the light transmitted through the subject from behind to illuminate the area of focus) may also be employed if appropriate. This technique may be relevant in photographically documenting deep tissue hemorrhage when bruising on the surface of the skin is less apparent.

Being able to accurately document and demonstrate key findings is essential in the field of forensic science. By minimizing glare and depicting the subject matter more accurately in resulting images, the photographic documentation becomes a more reliable and complete record. A forensic photographer in the field can use this simple technique to better visualize findings as applicable.

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### Forensic Photography, Flash Glare, ISO



## C31 On-Camera vs. Off-Camera Flash

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the utility of both the on-camera (pop-up) flash and off-camera flash to properly photograph postmortem examinations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing various scenarios in which the on-camera flash can provide a suitable alternative to an off-camera flash.

With insufficient ambient light and no external flash unit available, a forensic photographer will need to understand the on-camera flash's function and limitations when utilizing it as a primary light source. This presentation will discuss the appropriate usage of a camera's pop-up flash. Referencing the camera's owner's manual will indicate the maximum illumination distance for the built-in flash for any particular camera model. While this distance may minimize the on-camera flash's utility for crime scene photographs where wide-angle photographs are necessary, autopsy photography operates at much closer distances, typically within the range of the on-camera flash. For many years, professional forensic photographers have relied upon an off-camera flash connected with a flash cord to produce superior lighting compared to the camera's built-in flash. In most cases, newer camera models have an on-camera flash that is powerful enough to accommodate many autopsy photography scenarios. The majority of autopsy findings are photographed at close range and well within the maximum range of the pop-up flash and do not require the extra power provided by an external flash unit. By using the camera's on-camera flash, the photographer's other hand is free to stabilize the camera or position the scale and labels used for photographic documentation.

While many autopsy examination findings can be effectively photographed with the camera's on-camera flash, some scenarios will still benefit from an off-camera flash and flash cord. Glare remains a challenge for wet and reflective surfaces. In these instances, an off-camera flash allows for oblique lighting that will control the direction of reflected light. By placing the flash at a 45-degree angle from the subject matter, the strobe's light will bounce past the lens instead of directly in it. Without oblique lighting, the photographer may be forced to shoot at undesirable angles to allow the light to bounce past the lens, which may result in perspective distortion. Having a better understanding of these lighting factors can equip forensic photographers to effectively and efficiently provide photographic documentation during forensic examinations.

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### Flash, Photography, Pop-Up

## C32 Assessing Agreement Among Crime Scene Measurement Methods

Maria Christiene M. Lawas, MS\*, Federal Bureau of Investigation, Quantico, VA 22556; Susan Y. Williams, MS, Federal Bureau of Investigation, Quantico, VA 22556; Steven Jameson, BS, Federal Bureau of Investigation, Quantico, VA 22556; Anthony R. Gonzalez, MS, Federal Bureau of Investigation, Quantico, VA 22556; Paula Ernst, BS, Federal Bureau of Investigation, Quantico, VA 22556; Joseph Donfack, PhD, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will understand various technologies involved in crime scene reconstruction and how these different methods compare in terms of accuracy, closeness of the measurements to the ground truth value, when used for measurement.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by empowering evidence response teams to make informed decisions as to which method(s) are most suitable for a given crime scene.

A critical concern with crime scene documentation is the accuracy with which a crime scene can be reconstructed to better understand what occurred and to support any follow-on analyses. Technological advances have made it possible to have a wide array of measuring devices for the reconstruction and documentation of crime scenes, including laser-based and photogrammetric systems.<sup>1</sup> While all these devices are capable of accurate measurements, some may be easily influenced by environmental challenges (e.g., surface inclination, movement, humidity, temperature, vegetation, and distance) and human factors. As commercially available sensors and custom software have become cheaper and increasingly available, the more important question is how automated systems and hand-drawn methods compare in accuracy and whether this translates into additional benefits for investigators.

To compare crime scene documentation methods featuring different technologies, the accuracy of seven documentation methods as a function of measurement distance was assessed. Two methods that align well regardless of normality in their data will have better precision with respect to one another, as evidenced in relatively narrow confidence intervals and small measurement biases.<sup>2,3</sup> The relative accuracy of each documentation method was assessed with respect to a standard method, Total Station, from which measurements served as ground truth data.

The actual relative difference between measurements when compared to Total Station was small (less than a quarter of an inch). Measurements from FARO® Light Detection And Ranging (LiDAR) were the most comparable to those of Total Station, while a camera drone without the use of Ground Control Points (GCPs) were the least comparable. GCPs or a constrained reference scale were also found to be important in preventing increasing imprecision with increasing distance when measuring targets via drone and orthomosaic methods. Additionally, there were no statistical differences in the use of 2D (horizontal) or 3D (slope) measurement styles for the Total Station. As more measurement methods become available, and the need for training and validating new tools become a necessity, these results point to the importance of establishing a ground truth or known distance range on which crime scene measurement methods can be validated.

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### Crime Scene Reconstruction, Photogrammetry, Light Detection and Ranging (LiDAR)

## D1 The Development of a Fracture Risk Evaluation Method by Computer Simulation That Reproduces Pressure Distribution on Bone Due to Compression and Striking

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**Learning Overview:** After attending this presentation, attendees will have knowledge of the advanced fracture risk evaluation method using computer simulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an advanced fracture risk evaluation method using the Finite Element Method (FEM).

**Background:** In Japan, which faces a super-aged society, caregiver abuse of the elderly has become a social problem. Quantitative evaluation of external force received by a victim is necessary as a means to prove the murder of the perpetrator when an abuse case occurs. In recent years, it has become possible to evaluate fracture risk considering bone shape and bone density distribution by using computer simulation. Furthermore, a method has been developed to analyze the pressure distribution with high accuracy when the external force applied to the bone is static. On the other hand, even when the external force applied to the bone is dynamic, a uniform average pressure is used as the external force condition in the analysis, even though the pressure distribution varies greatly depending on the tip shape of the blunt instrument. This may reduce the accuracy of the analysis results (fracture risk evaluation).

**Purpose and Experimental Outline:** This study proposed a fracture risk evaluation method by computer simulation that reproduces the pressure distribution on the bone due to compression and striking, and its usefulness was verified by using it for two cases.

**Case 1: Ribs of the Elderly Undergo Static External Forces During Caregiving.** When static external force acts on the human body, fracture risk evaluation that reproduces the pressure distribution on bone can be performed by the following procedure: (1) the external force, which is applied to the human body using the pressure textile sensor, is measured. The pressure distribution applied to the bone is estimated by comparing the measured external force with the bone image of the part to which the external force is applied, and (2) the fracture risk is evaluated by adding the estimated pressure distribution to the bone model on FEM.

**Case 2: An Infant's Skull Receives a Dynamic External Force When Hit by a Blunt Instrument.** When a dynamic external force acts on the human body, fracture risk evaluation that reproduces the pressure distribution on the bone can be performed by the following procedure: (1) a falling weight experiment that reproduces how a steel ball (blunt instrument) collides with the head is performed. The maximum load at the time of collision is measured with a load cell, and the maximum pressure distribution is measured with pressure-sensitive paper; and (2) the fracture risk is evaluated by adding the pressure distribution estimated by the load cell and pressure-sensitive paper to the bone FEM model.

**Results and Discussion:** It was shown that a bone fracture risk evaluation method by computer simulation, which reproduces the pressure distribution, can be performed by measuring the maximum pressure distribution applied to the bone using the pressure textile sensor in the case of static external force or pressure-sensitive paper in the case of dynamic external force, depending on the load speed of the human body. It was also shown that the conventional analysis method in which the average pressure was applied to the entire external load area of the bone calculated the fracture risk lower than that of the method proposed in this study.

The human body receives dynamic external force not only when it is hit with a blunt instrument or a fist, but also when it crashes, falls, and collides with the floor or road surface. It is considered that the fracture risk evaluation method developed here that reproduces the pressure distribution on the bone by computer simulation would be also a useful tool for evaluating the risk of falling fractures in the elderly and the interpersonal safety of caregiving robots.

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**Fracture Risk Evaluation, FEM Analysis, Pressure Distribution**

## D2 A Novel, Data-Driven Approach to the Classification of Bloodstain Patterns

Tong Zou, MS\*, University of California, Irvine, Irvine, CA 92617; Hal S. Stern, PhD\*, University of California - Dept of Statistics, Irvine, CA 92697

**Learning Overview:** Attendees in this session will learn about a data-driven machine learning method for classifying bloodstain patterns produced by different mechanisms or event types.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a data-driven machine learning method that integrates complete information from the bloodstain pattern. The method is objective in the sense of not relying on expert opinion to identify the key features that correspond to a particular blood-producing mechanism or event. Preliminary results suggest effective classification of mechanism based on image analysis of bloodstain patterns.

Bloodstain pattern analysis is the study of bloodstains at a crime scene with the purpose of drawing inference about the crime. A typical objective for bloodstain pattern analysis is to identify the cause/mechanism that produced the pattern. Examples of possible blood-producing mechanisms or events include blunt impact, cast-off, and gunshot. Analysts of bloodstain patterns frequently rely on measurements of the size, direction, shape, and spatial distribution of the stains to classify the patterns.

In recent years, bloodstain pattern analysis and other forensic disciplines have drawn considerable scrutiny. A comprehensive 2009 National Academy of Sciences Report questioned the opinions of bloodstain pattern experts as more subjective than scientific and also questioned the reliability of the traditional methods used for analyses.<sup>1</sup> One result of this scrutiny is an increasing interest trend in the forensic science research community to build models to classify bloodstain patterns that can address the concerns that have been expressed. Recent studies have achieved notable classification accuracy by using machine learning models based on researcher-defined features of the stains and patterns.<sup>2,3</sup> However, there are two limitations associated with these methods: (1) they rely on an ellipse representation of each bloodstain and thus may not work well for all categories of bloodstain patterns; and (2) although pattern-level features may be defined that allow us to differentiate certain categories of bloodstain pattern, there is no guarantee they are sufficient for all possible categories because the reliance on researcher-defined features can be limiting.

This presentation reports preliminary results on a novel method to address these two limitations of recently reported machine learning approaches. First, as noted above, the traditional ellipse representation of stains, which requires each stain be approximated well by an ellipse and omits non-elliptical shaped stains, may fail to characterize some types of patterns. This concern is addressed by generalizing the ellipse representation to allow for each stain to be approximated by multiple ellipses through an ellipse segmentation algorithm. Consequently, overlapping stains and stains with irregular shapes can be included to enhance the analysis. The second limitation is the reliance on expert-defined, pattern-level features that are believed to serve as a suitable summary of the distribution of the ellipses in the pattern. A concern is that some important information regarding the distribution of the ellipses is lost when relying only on these features. Instead of designing possible features based on prior knowledge, a flexible approach is applied that summarizes the information in the pattern (i.e., the information about the distribution of the stains) using a statistical concept known as the characteristic function. Then, by defining a distance metric between any two characteristic functions, flexible machine-learning classification methods (e.g., support vector machine) were able to be applied to bloodstain patterns. This approach transfers complete information about the distribution of stains in a pattern to a classifier without defining any explicit summary features. To illustrate the potential of this method, this study conducted a pilot experiment with two sets of bloodstain patterns collected from different laboratory apparatuses. Preliminary results have shown superior performance of this method over previous ones in classifying bloodstain patterns produced by different mechanisms.

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### Bloodstain Pattern Analysis, Image Analysis, Classification

## **D3 The Determination of Delta-9-Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), and Total THC in Seized Cannabis (Hemp and Marijuana) Samples by Liquid Chromatography With Ultraviolet Detection (LC-UV)**

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**Learning Overview:** The goal of this presentation is to summarize the evaluation of an extraction and LC-UV absorbance detection method for measuring delta-9 ( $\Delta^9$ )-THC, THCA, and total THC in seized cannabis (marijuana and hemp) samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic scientists with an analytical procedure and quantitative method to confidently distinguish seized cannabis samples as either legal hemp or illegal marijuana.

The medicinal and recreational use of cannabis (hemp and marijuana) and cannabis-derived products continues to increase across the United States. As the industry grows, so does the need for reliable differentiation between legal and illegal products. The Agriculture Improvement Act of 2018 no longer defines hemp and its derivatives as Schedule I substances, while marijuana remains federally illegal. In addition, hemp was defined as cannabis materials having a  $\Delta^9$ -THC concentration of no more than 0.3 % on a dry weight basis. Prior to the legalization of hemp, forensic laboratories were required to only identify the presence of  $\Delta^9$ -THC through a qualitative procedure, including microscopic analysis, colorimetric test, and Gas Chromatography/Mass Spectrometry (GC/MS) detection using matching retention times and mass spectrum to pure  $\Delta^9$ -THC reference standards. Forensic laboratories are required now to distinguish seized cannabis samples as either legal hemp or illegal marijuana, leading to the need for new sample preparation protocols and standardized analytical detection methods.

The National Institute of Standards and Technology (NIST) has conducted multiple method development studies of an LC-UV detection method. Prior to analysis, cannabis samples were solvent extracted with the addition of 20mL of methanol, mixing, and centrifugation. Following a 10-fold and 100-fold dilution, the extracts are filtered using a 0.45 $\mu$ m Polytetrafluoroethylene (PTFE) syringe filter into an autosampler vial. This presentation will summarize the initial evaluation of this extraction protocol and LC-UV method for the determination of  $\Delta^9$ -THC, THCA, and total THC in hemp reference samples and seized cannabis (hemp and marijuana) samples. In the case of United Kingdom reference samples, comparison was made to their Certificate of Analysis to ensure the accuracy and precise of the LC-UV methods. Overall, the NIST measurements were in good agreement for  $\Delta^9$ -THC, THCA, and total THC mass fraction (%) values in four different hemp samples in the multiple method development studies. In the case of seized cannabis samples, the LC-UV values were in good agreement with the other analytical methods developed at NIST, including GC/MS and LC couple to Tandem Mass Spectrometry (MS/MS). A Cannabis Quality Assurance Program (CannaQAP) has been developed and the development of a hemp Reference Material (RM) has been started aimed at reducing discrepancies in analytical measurements within the forensic community. The same methods summarized here are used for the initial screening of CannaQAP and RM samples.

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### **Liquid Chromatography, Seized Samples, Cannabis**

## D4 A Hyperspectral Analysis of Fabrics Submerged in the Indian Ocean: An Innovative Way to Aid in the Estimation of the Time Human Remains Have Spent in Water

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**Learning Overview:** After attending this presentation, attendees will understand the ways in which Hyperspectral Imaging (HSI) may be utilized in an aquatic forensic setting to age different fabrics (cotton, neoprene, satin, and velvet) following submergence in a marine environment for a period of one to six months. The outcome of this research identifies how the reflectance changes through time and with which fabrics this methodology can be successfully applied.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential usage of HSI in aquatic crime scene analysis. Furthermore, this research will contribute toward increasing the number of tools forensic investigators have at their disposal for increasing the accuracy of the minimum Postmortem Submergence Interval (minPMSI) of clothed human remains in a marine environment.

The estimation of the time since death, minimum Postmortem Interval (minPMI), is a crucial aspect of a forensic investigation. In an aquatic environment, this process is particularly challenging due to the complexity of the corpse's decomposition process and the many impacting environmental factors. Furthermore, there is a general paucity of research in this field. Recently, the use of clothing recovered alongside a corpse in aquatic crime scenes has come under focus for their potential in the estimation of the time spent in water. It is likely for clothing to be present and to be colonized by biofouling organisms (e.g., barnacles), which can be used to estimate the time the victim has spent in water. However, thus far, biological/zoological-based estimation methods are the only avenues that have been explored for aging clothing in an underwater context. This research is the first to focus on the use of HSI for aging fabrics, considering the modification of their optical properties as a result of exposure to a marine environment.

This research focused on four common fabrics: cotton, neoprene, velvet, and satin, which were submerged in the Indian Ocean off the coast of Perth, Western Australia, for a period of one to six months. The aims of this research were: (1) to explore and quantify the optical properties of the fabrics by comparing spectral profiles and how they change due to marine exposure; and (2) to determine whether this information can offer support in estimating the minPMSI of clothed human remains recovered from a marine environment. Fabric samples were used to generate spectral profiles for all the submerged fabrics and controls ( $n=112$ ). Spectral data was collected at 76 wavelengths between 400-1,020nm using GenASIs® Hyperspectral Imaging Instrument and, using the GenASIs® Spectraview® image analysis software, three pixels were selected from each sample for analysis. The analysis focused on comparing the average spectral reflectance within and between the fabric samples at monthly intervals to determine whether a significant change occurred as a result of marine exposure.

Cotton demonstrated the most significant changes as a result of exposure. Physically, the material became highly degraded and, by six months, was structurally compromised. A strong negative regression in the average spectral reflectance was recorded over the six months. There was no significant regression trend identified for satin, neoprene, or velvet. Satin demonstrated notable changes with a significant difference in the average spectral reflectance occurring between the control samples and the submerged samples, but with little differences between the submerged samples. Neoprene demonstrated minimal significant changes, with months one to four showing similar profiles to the controls and some significant differences in months five and six. This result suggests that a more longitudinal study may yield more data from neoprene. Furthermore, neoprene was also the least physically altered fabric out of the four, showing minimal degradation as a result of exposure to the marine environment. Velvet demonstrated no significant change in average spectral reflectance as a result of aquatic exposure. Overall, results show that the HSI approach can be successful when used on cotton and satin in the first months of submergence, while HSI highlights the changes in neoprene after at least four months of submergence. With regard to velvet, the HSI method is not suggested.

This research establishes the potential of a non-invasive technique to provide age estimations of fabrics recovered in aquatic forensic investigations, useful when fabrics are not colonized by fauna or—on colonized fabrics—to provide more information, in association with the zoological method.

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### Aquatic, Hyperspectral Imaging, Postmortem Submersion Interval



## D5 Forensic Investigative Issues in a Fireworks Production Factory Explosion

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**Learning Overview:** After attending this presentation, attendees will understand the importance of: (1) a multidisciplinary expertise in the reconstruction of the dynamics of explosive events, and (2) a thorough study of the lesions detected in relation to the injury mechanisms produced in order to ascertain the cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the important contribution of the forensic investigations in particular cases, such as explosions, to the determination of manner and cause of death, when properly combined to the information provided by different professional figures.

Fireworks are used all over the world for both public and private celebrative events. Although they are commonly considered safe items, accidents are not infrequent, especially when they are managed by inexperienced people. The most relevant damages occur within production factories, either for the lack of safety regulations or the employment of untrained staff, with a subsequent high social and economic impact in terms of morbidity and mortality. Fireworks-related accidents are mostly accidental, although sometimes, depending on the circumstantial data and judicial investigations, an intentional nature must be suspected. In such a context, an adequate engineer expertise becomes of utmost importance to shed light on the dynamic of the event. When such information proves compatible with the forensic investigations—site and position of the bodies, lesions detected and compatibility with the injury mechanisms produced by the explosion—as well as with the circumstantial data provided, the intentional or accidental nature of both the explosion and deaths can be ascertained.

The importance of such aspects is highlighted in the present work, which reports the case of a fireworks production factory explosion in which seven people became involved: four of them, three of which engaged in the installation of sliding gates to the factory buildings, died immediately; a man, engaged in the same task, died while transported to the nearest hospital; the remaining two people survived. According to the preliminary judicial investigations, contracts had lately gone on between the factory's owner and a worker hired without a regular contract. For this reason, an intentional explosion was suspected, and an engineer survey was requested. The factory consisted of a total of 16 buildings: buildings number 6 and 7 were completely destroyed by the explosion, and building number 8 also caught fire; all the other buildings were affected by minor damages. The corpses of two of the victims were found nearby the buildings number 6 and 7; several body parts of a third victim were found spread in the area surrounding the same buildings and collected; the fourth victim's corpse was found quite completely charred in proximity of building number 8. Following the survey of the fire investigative unit engineer, the dynamics of the explosion were elucidated: a first explosion occurred in building number 7, where four workers were engaged in the installation of a sliding gate, due to a deflagration caused by welding sparks in an area with a combustible-oxidizing atmosphere. The ignited atmosphere thus acted as a fuse for a domino effect, mainly affecting buildings number 6 and 8 (used, respectively, as fireworks deposit and a fireworks production station), which were very close to building number 7. The positions of the bodies and the lesions detected were compatible with the described dynamic, with four victims dead from the explosive effects and one from fire-related charring. All engineering findings were compatible with an accidental explosion occurring during welding activities for the installation of sliding gates; the intentional hypothesis was then rejected.

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### Fireworks, Factory Explosion, Multidisciplinary Investigations

## D6 Monte Carlo Uncertainty Analysis for the Measurement of Forensic Glass Using Neutron Activation Analysis and Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS)

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**Learning Overview:** The goal of this presentation is to highlight the importance of proper handling of uncertainty analyses for LA-ICP/MS and to show the steps being taken to develop the procedures for certifying a new glass Standard Reference Material (SRM) for the forensics community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by empowering analysts and technicians to understand their instrument's and technique's uncertainties and enable them to confirm whether their procedures are adequately incorporating uncertainty propagations.

The ubiquity of glass makes it a commonly encountered type of forensic trace evidence. Elemental analysis using sensitive analytical techniques, such as LA-ICP/MS, is routinely used to compare the chemical composition of glass specimens submitted in casework. The forensic community uses several National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM 1831, SRM 610, SRM 612, and SRM 614) and international glass reference materials (Float Glass Standard 1 [FGS1] and FGS2) for quality control and external calibration. However, the community currently relies on consensus or reference (i.e., not certified) concentration values for all elements in the FGS glasses and some elements, particularly the trace elements, in the NIST SRMs. Additionally, many of the certified element concentration values in the NIST SRM certificates were determined over 20 years ago. Finally, several glass reference materials are currently low in stock and will need to be replaced within the next decade. To address these gaps, the elements in the NIST SRMs and three newly designed float glass reference materials produced by Corning<sup>®</sup>, Inc. (in collaboration with Florida International University) will be evaluated with modern methodology. Neutron Activation Analysis (NAA) and LA-ICP/MS will be used to obtain statistically robust concentration values at the bulk and micro sampling scale, respectively.

To estimate the uncertainty in NAA and LA-ICP/MS measurements, a rigorous Monte Carlo-based method has been developed. The Monte Carlo uncertainty analysis method complements and expands upon the Guide to Expression of Uncertainty in Measurement (GUM) method. The Monte Carlo method accounts for all correlations introduced through common input variables (e.g., standards) and for the dark uncertainty between measurements. An R script that conducts the Monte Carlo uncertainty calculations for NAA has been developed. The uncertainty budget analysis of NAA revealed that an improvement in uncertainties could be achieved by reducing correlations in the standards (a four-fold improvement in uncertainties) and by increasing the number of wafers analyzed. An R script that combines data reduction of the raw LA-ICP/MS signal, quantitation using single-point external calibration, and a Monte Carlo uncertainty analysis has been written for LA-ICP/MS measurements. The uncertainty budget analysis will aid in evaluating LA-ICP/MS as a potential analytical tool for the certification of major, minor, and trace elements in glass at NIST. Additionally, the uncertainty budget analysis will aid in determining a practical number of fragments and replicate measurements per fragment for the certification of elements in the three new float glass reference materials for the forensic science community. The results for the Monte Carlo uncertainty budget analysis of NAA and LA-ICP/MS will be presented.

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### Glass, Trace Elements, Glass Standard



## **D7    Portable Dynamic Vapor Microextraction for Forensic and Environmental Field Studies**

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**WITHDRAWN**

## D8 A Fatal Hyperbaric Treatment Explosion Investigation Incorporating Engineering Simulations With Verification and Validation

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**Learning Overview:** After attending this presentation, attendees will understand some of the principles in using engineering simulations in support of forensic investigations, how to apply Verification & Validation (V&V), some of the specific strengths and pitfalls of Finite Element Analysis (FEA) and Computational Fluid Dynamics (CFD), and how a variation of an established medical device used for a different application can result in an unintended fatal hazard with attendant liability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the use of engineering simulation, coupled with traditional engineering methods, to assess a catastrophic medical treatment event and demonstrate a potential low-cost mitigation. The engineering simulations not only allow for a more detailed examination of complex multi-physics events, it provides tools to clearly communicate the results to a lay audience. In forensic applications, the use of V&V methods could dictate whether simulations are admissible.

Engineering simulations have been an established tool for decades in the design of equipment, including medical devices. Investigating an equipment incident generally involves the equipment failing in some manner. Conventional calculations and simplified simulations are often sufficient for design work because the design intent is to establish a significantly safe design margin between the operating conditions and likely failure modes.<sup>1</sup> Failures, on the other hand, often involve non-linear or dynamic mechanisms well outside normal operating parameters. Additional knowledge and skill are needed to investigate equipment failure modes and assess the factors that led to the failure.

Engineering simulations face an additional hurdle in forensic applications. While illustrations and animations are generally treated by the courts as an extension of the expert's opinion, the static or animated images from engineering simulations are a result of intensive, complex calculations independent of the investigator.<sup>2</sup> This independence is a strength when properly coupled with other means, such as traditional engineering calculations, laboratory experiments, and other accepted methods of investigation. V&V provides a methodology to establish this linkage.<sup>3,4</sup>

The independence of simulations from the investigator is also a potential liability. Simulations can be seen as substantive evidence, not simply "illustrations." As such, they are subject to scrutiny similar to more traditional scientific tests. This means simulations could be challenged per the scientific evidence admissibility standards of the jurisdiction. The simulation's proponent must establish that the evidence is "based upon sufficient facts or data," is "the product of reliable principles and methods," and that the supporting expert witness "applied principles and methods reliably" when creating or using the simulation.<sup>2</sup> For these reasons, the investigator needs to not only understand the fundamental physics being modeled but also the V&V methods applied to the investigation to establish admissibility of the simulations.

This report will present a case study of a catastrophic hyperbaric treatment incident. The engineering codes and standards for medical hyperbaric systems in the United States is the American Society of Mechanical Engineers (ASME) Safety Standard for Pressure Vessels for Human Occupancy, designated PVHO-1.<sup>5</sup> The chamber in question, however, was for treating horses and, therefore, was excluded from the scope of the standard. A horse became agitated while in a pressurized metal chamber with an enhanced oxygen atmosphere. Key aspects of hyperbaric treatment for horses are not considered in PVHO-1. These key aspects led to the incident's cause of origin. The forensic engineer investigating the incident used FEA and CFD engineering simulation, coupled with ergonomic factors and Failure Modes and Effects Analysis (FMEA), to assess the root cause, assess liability, and develop a mitigation option.

It is recommended that investigators of equipment-related incidents learn the principles of applying engineering simulations such as FEA and CFD as well as how to determine if the simulations were properly established using V&V.

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### Simulation, Fire, Fatal

## D9 Post-Blast Explosives Attribution

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the sample collection, sample processing, and instrumental analysis techniques involved in identifying chemical attribution signatures from trace post-blast explosive residue and potential data analysis methods to determine the source of an explosive material from the identified signatures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating proof-of-concept results on a novel approach to attribution of explosives to their sources post-blast.

Forensic science practitioners in both domestic and Department of Defense organizations are often called upon to build cases and attribute crimes using trace evidence remaining at a crime scene. The ultimate goal of these investigations is to associate a crime with a suspect or suspects in order to prevent further attacks through exclusion, exoneration, arrest, and criminal prosecution of potential perpetrators. However, fundamental questions remain regarding evaluation and interpretation of trace evidence for post-blast explosives attribution. The explosive charge is an attractive component for attribution in crimes involving explosives as it is key to the functioning of the device, and there are limited pathways for acquisition. However, unlike pre-blast attribution where signatures from explosive manufacturer reference samples can be compared with recovered samples, there is currently no capability to link the explosive charge to its source via post-blast trace residues. Attributing the explosive post-blast is a challenge because very little explosive material remains after detonation, which limits the analytical techniques that can be used. The purpose of this study is to determine whether pre-blast attribution signatures are preserved after detonation, and whether they can be recovered from a blast site and measured at a detectable level.

In this study, a preliminary field test was conducted to recover post-blast explosive samples from controlled detonations of multiple explosive materials including RDX, TNT, and Ammonium Nitrate-Aluminum (AN-AL). Samples were processed according to adapted methods to extract both the explosive compound of interest, as well as other chemical components that could potentially serve as signatures for attribution.<sup>1,2</sup> Samples were subsequently analyzed via multiple analytical techniques including High-Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) for polar and non-polar small molecules, Internal Positive Control/Mass Spectrometry (ICP/MS) for trace elements, and Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS) or Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) for isotope ratios of carbon, nitrogen, and oxygen. Preliminary results have shown promise that carbon, nitrogen, and oxygen isotope ratios remain consistent pre- and post-detonation and thus could be relevant for source attribution. For RDX, the average difference in isotope ratios between pre- and post-detonation is 0.33 ‰ (max difference=0.58 ‰) for carbon and 0.2 ‰ (max difference=1.20 ‰) for nitrogen. For TNT, the average difference is 0.2 ‰ (max difference=1.36 ‰) for carbon and 0.5 ‰ (max difference=1.77 ‰) for nitrogen. Finally, for AN-AL, the average difference is 0.13 ‰ (max difference=0.54 ‰) for oxygen and 0.10 ‰ (max difference=0.37 ‰) for nitrogen. The analytical measurement uncertainties (standard deviation of repeated analyses of isotopic reference standards) were less than or equal to 0.51 ‰ across all isotopes and material types. These results indicate that isotope ratio signatures of explosive compounds look to have been preserved after detonation in this small sample set. With this proof-of-concept study, the forensic community will benefit from a novel approach to attribute explosives after detonation.

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### Attribution, Explosives, Post-Blast

## D10 Examining the Repeatability and Reproducibility of the Heat Release Rate (HRR) From Upholstered Furniture

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**Learning Overview:** The goals of this presentation are: (1) to understand the potential use of HRRs in the investigation and analysis of fire incidents; (2) to understand the reproducibility and uncertainties of the determination of HRR from oxygen consumption calorimeters; and (3) to provide insight to fire investigators and engineers on the repeatability and the limitations of the HRRs and total heat released generated by burning upholstered furnishings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that good agreement was found for both the repeatability of the HRRs and reproducibility of the results between the three full-scale oxygen consumption calorimeters used in the study. Care must be used when applying HRRs to the investigation of fires.

Heat release rate is one of the most important input variables for use in numerically simulating a fire.<sup>1</sup> National Fire Protection Association (NFPA) 921, Guide for Fire and Explosion Investigations, considers mathematical modeling techniques as a means to provide the fire investigator with tools for testing hypotheses regarding the origin and cause of the fire and the cause of the resulting damage to property or injury to people. NFPA 921 cautions that “mathematical modeling, whether simplified hand calculations or computer fire models, has inherent limitations and assumptions that should be considered. Models generally rely upon empirical data and are validated via comparison with other empirical data. Care must be taken to assure that the model is being used with due regard for limitations, assumptions, and validation.”<sup>2</sup> The National Institute of Justice Technology Working Group Operational Requirements (TWG OR) for Fire Investigation include the need for adequate materials property data inputs for accurate computer models.<sup>3</sup>

A better understanding of the measurement of heat release rates will provide a basis for the improved use of heat release rates in fire investigations. A starting point was the examination of the capabilities of the full-scale calorimeters used to measure heat release rate. Underwriters Laboratories Firefighter Safety Research Institute (UL FSRI) partnered with the Bureau of Alcohol, Tobacco and Firearms (ATF), the National Institute of Standards and Technology (NIST), and UL LLC to conduct a series of natural gas-fueled burner experiments as well as burning replicates of similar upholstered chairs to examine both the repeatability of the experiments at each laboratory and the reproducibility between the three laboratories.

Natural gas-fueled burners were positioned under the oxygen consumption calorimeter. The three different heat release rates selected for examination were 100kW, 500kW, and 1,000kW. Three replicate measurements were made for each heat release rate with each calorimeter. The flow of natural gas was measured with a mass flow controller. The heat release rate was predicted based on the heat content of the natural gas and the mass flow of the natural gas. The predicted heat release rate served as the comparison value for the heat release rate determined with the calorimeter. These experiments provided insight into the uncertainty and repeatability of the oxygen consumption calorimeters as well as an examination of the reproducibility of these baseline HRR measurements.

Fire investigators rarely investigate a fire that involves a natural gas-fueled laboratory burner. Therefore, additional heat release rate experiments were conducted with three types of upholstered chairs. Replicate heat release rate measurements were made with each chair to examine the repeatability of the burning chairs as well as to examine the reproducibility of the heat release rate measurements between the laboratories. Additional heat release rate experiments with additional types of upholstered chairs and sofas were conducted with the UL calorimeter.

*Results of each of the sets of measurements will be presented. This research was supported by United States Department of Justice, National Institute of Justice Grant Award, 2017-DN-BX-0163.*

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### Heat Release Rate, Fire Investigation, Upholstered Furniture

## D11 Using Object Detection to Obtain Flame Height From Experimental Video and Evaluate Predictive Models

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**Learning Overview:** After attending this presentation, attendees will: (1) be familiar with potential uses of flame height in the investigation and analysis of fire incidents; (2) recognize how a machine learning model for object detection can be used to obtain flame height data from experimental video; and (3) understand the capabilities and limitations of algebraic formulas commonly used to predict flame height.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining how, through the application of an object detection machine learning model, flame height data were collected from experimental video of free burn and compartment fires. The data were then used to assess predictive algorithms designed to estimate flame height. The discrepancy between the predicted and measured flame heights tended to be larger for fire scenarios that involved more complex fuels.

Over the years, researchers have developed a variety of techniques to estimate certain characteristics of a fire and its surrounding environment. Many fire investigation textbooks and engineering handbooks contain detailed information regarding algebraic formulas derived from empirical correlations that are used to predict different aspects of a fire.<sup>1,2</sup> Furthermore, training programs offered by the International Association of Arson Investigators describe a similar set of algebraic equations that can be used to estimate fire plume temperature, flame height, hot gas layer temperature, radiant heat flux from a fire source to a target fuel, time to compartment flashover, and other quantities for a given fire scenario.<sup>3</sup>

The vast majority of these empirically based algorithms are based on data collected from experiments involving gas burner and/or liquid pool fires, which typically have high levels of repeatability and can often be defined by the two-dimensional planar surface from which the fuel is expelled.<sup>2,4</sup> However, many fire scenarios involve fuel sources that are more complex than these laboratory fuels, such as a residential structure fire fueled by furnishings, appliances, and other items located inside the dwelling. These types of fuels are often composed of synthetic materials that melt, drip, and/or flow when heated and can emit fuel from multiple surfaces at any given time during the fire. Therefore, the flame geometry from a fire with a laboratory fuel may not accurately represent the flame geometry from a fire with a more complex fuel.

Over 100 full-scale experiments were conducted to examine the performance of numerous predictive algorithms for fire scenarios that used a natural gas burner, upholstered sofa, or upholstered chair as fuel. Fires from these fuels were studied under free burn conditions as well as inside a compartment with interior dimensions of 3.7m long by 3.7m wide by 2.4m high. A single ventilation opening was present in the form of a 2.0m high by 0.9m wide doorway located on the front side of the compartment. To study the effect of fuel location on fire dynamics, fuels were positioned at various locations in the compartment during the fire experiments. Additionally, experiments were performed with the compartment door in both the open and closed positions to evaluate predictive methods under conditions with different ventilation patterns.

Historically, researchers have measured flame height through various methods that include analysis of still images or video; analysis of infrared-imaged photos or video; a 500°C average centerline temperature criterion; and visual estimation by an individual.<sup>5,6</sup> For the majority of these experiments, flame height data were obtained through the analysis of recorded video. More specifically, a machine learning model was successfully trained and deployed to identify the fuel source, flame(s), and reference markers in the still frames of each experimental video. Knowing the vertical distance between each reference marker, the number of pixels per meter was computed for each analyzed video. Then, the object detection model was used to identify any flame(s) in each video frame, and the number of pixels between the tip and base of the flame was computed and converted to meters.

Experimental flame height data were compared to predicted values from empirical correlations. Results suggest that the magnitude of the difference between predicted and measured flame heights may depend on the fuel location within the compartment. Additionally, discrepancies between predicted and measured flame heights tended to be larger for scenarios that involved a more complex fuel, such as the upholstered chair or upholstered sofa utilized for these experiments.

As a result of this project, a new method has been developed to extract flame height data from recorded video. This procedure could be utilized by researchers and engineers in the future to effectively measure flame height during fire scenarios. Furthermore, experimental flame height data were compared to the values predicted by commonly used algebraic formulas. Results from this study provide fire investigators and engineers with credible scientific information based on data from full-scale fire experiments to better understand the capabilities and limitations of algorithms used to calculate fire phenomena.

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### Fire Investigation, Flame Height, Predictive Fire Models

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## **D12 Carbon Monoxide Poisoning in a Heavy Truck: An Investigation of Unknown Causes of a Fatal Crash With Fuel-Fed Fire**

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**Learning Overview:** This investigation explores the case of a Class 8 tractor-trailer that left the roadway for no apparent reason. The investigation included only partial evaluation of the exhaust system integrity. Several steps of the proposed investigation were disallowed by the client, but will be discussed in detail. After attending this presentation, attendees will be better equipped to recognize and analyze cases involving, or potentially involving, Carbon Monoxide (CO) poisoning in moving vehicles, including exhaust system component inspection procedures and testing with the objective of establishing design, materials, or maintenance defects. Professional conflicts with clients over authorization for proper scientific work is also discussed.

**Impact on the Forensic Science Community:** This presentation will impact engineers, scientists, criminologists, attorneys, pathologists, police, and others involved in the forensic aspects of traffic accident investigation and reconstruction and vehicle fire investigation, especially involving heavy trucks.

Diesel engine exhaust gas contains several constituents immediately harmful to man: CO, Hydrocarbons (HC), and aldehydes. CO can rapidly cause headaches, dizziness, lethargy, and loss of consciousness. Engines with defective exhaust systems combined with defective fresh air intake design can cause CO to easily accumulate in one popular heavy truck cab. A driver with no alcohol or drugs in their system was operating one of these day cab tractor-trailers on a familiar freeway when, for no apparent reason, the vehicle drifted to the right off the roadway, struck a fixed object, ruptured the fuel tanks, and ignited. The restrained driver showed no impact trauma, and witnesses claimed no attempt to self-extricate or even cry out. Postmortem blood analysis revealed the driver's carboxyhemoglobin level was extremely high, consistent with unconsciousness leading to the loss of control and collision, as well as the inability to remove the restraints and self-extricate.

Initial investigation of the subject tractor make and model revealed a history of exhaust system component failures, consistent with the subject crash. Evaluation of an exemplar tractor revealed that the cab makeup air intake aperture was located under the hood near the area of exhaust system component failures and that there was a direct open path for the transfer of air containing leaking exhaust gas into the tractor cab. An initial inspection and testing of the heavily burned subject tractor and exhaust system components was completed. Per common experience, fire destruction of seals and gaskets made it impossible to conclusively prove an exhaust leak simply by inspection. Unfortunately, the client refused to allow testing of an exemplar vehicle to scientifically replicate the potential failure that caused the CO contamination of cab air via the defectively designed fresh air intake. The investigation of possible CO sources and gas conduction paths will be discussed and analyzed. This presentation will detail the investigative actions taken and those that were proposed in order to isolate the CO debilitation of the driver that most likely precipitated the crash, but was disallowed. The ethical and professional dilemmas created by client refusal to support proper forensic scientific investigation will also be discussed.

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### **Carbon Monoxide Poisoning, Heavy Truck Fire, Defective Fresh Air Intake**



## D13 A Heavy Truck Fuel-Fed Fire: Vehicle Design Failures Causing Predictable Fatality in Otherwise-Survivable Conditions

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**Learning Overview:** This presentation explores the case of a heavy tractor-trailer fire that killed an uninjured, restrained driver. The investigation included evaluation of the exhaust system integrity, defective axle dislocation, dangerous fuel tank placement, and alternative designs that would have prevented these failures. After attending this presentation, attendees will be better equipped to recognize and analyze heavy truck crashes and fires with the objective of establishing accurate cause and effect for occupant injury and property damage.

**Impact on the Forensic Science Community:** This presentation will impact engineers, scientists, criminologists, attorneys, pathologists, police, and others involved in the forensic aspects of traffic accident investigation and reconstruction and vehicle fire investigation, especially in heavy trucks.

In this case study, a restrained driver was operating a day cab tractor-trailer on a familiar, flat, straight freeway when, for unknown reasons, the vehicle drifted to the right off the roadway, sideswiped a tree, broke off the front axle, ruptured the side-saddle diesel fuel tanks, and burned. The driver had no collision-related injuries, yet died due to the fuel-fed fire. Postmortem blood analysis revealed the driver's carboxyhemoglobin level was extremely high, consistent with pre-crash loss of consciousness, which is covered in a separate presentation. Initial investigation of the subject truck revealed that in the relatively low-velocity offset front collision, the poorly designed front axle of the tractor had sheared off and was displaced rearward, allowing the front tires and wheels to rupture the exposed fuel tanks. This spewed a large volume of atomized fuel, which ignited and fatally burned the uninjured driver. Several hypotheses regarding the cause of vehicle axle separation, fuel tank rupture, and resulting fire were investigated, evaluated, and tested.

The late-model subject truck had virtually identical fuel tank placement as trucks produced in the early 1940s. Diesel fuel tank placement and guarding varies widely, depending on the size of the truck as well as the safety regulations in the country of operation or origination. In many areas of Europe and Asia, such heavy truck diesel fuel systems are guarded and/or completely relocated for impact protection. Fuel leakage prevention and ignition countermeasures had been developed over 50 years ago to reduce fire risks in heavy trucks. However, the heaviest class 7 and 8 trucks and vocational vehicles operated in the United States typically utilize large-volume, unguarded side-saddle type fuel tanks attached outside one or both frame rails, without check valves or any means to prevent impact-related leakage. Side-saddle tanks are usually in close proximity to the cab and any occupant egress paths, as were the tanks in this case. Such exposed fuel tanks are generally adjacent to unguarded batteries and cables mounted outside the frame rails. This creates an extraordinary proximity of very vulnerable, constantly exposed large volumes of fuel adjacent to high-energy ignition sources that are subject to collisions by other vehicles and fixed objects, which also are often adjacent to vehicle occupants. The exposed, dangerous location of such heavy truck fuel tanks clearly violates many long-established principles of reasonably safe vehicle fuel system design.

Crashworthy fuel system designs have been determined by crash test research published since at least the early 1960s that discovered dangerous flaws in ground vehicle and aircraft fuel tank location and materials. Research experience includes participation in the Department of Defense and National Highway Traffic Safety Administration (NHTSA) ground vehicle and aircraft fuel system safety research in the 1970s and 1980s. This was performed per the military Crash Survival Design Guide and Federal Motor Vehicle Safety Standard (FMVSS) 301, as well as defect investigation crash tests on the Ford® Pinto®, General Motors® side-saddle tank pickups, school buses, and several other vehicles that led to safety recalls. The FMVSS, including 301 for fuel system integrity, tend to be significantly less robust or non-existent for larger vehicles with Gross Vehicle Weight Rating (GVWR) over 10,000 pounds. There are no crash-related fuel system safety standards for heavy trucks, which is made worse by the myth that "diesel fuel does not burn" in collisions.

The current investigation included the fabrication, installation, and testing of a relocated, far safer, equal capacity fuel tank on an exemplar day cab tractor matching the subject tractor, based on principles established in the 1960s and 1970s. This study also discovered production alternative design fuel tanks and tank inserts that could have prevented the subject fire. The investigation, analysis, and testing involved in this case will be presented and discussed.

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### Heavy Truck Fire, Truck Fuel System Defects, Heavy Truck Crashworthiness

## D14 Reconstructing Energy Data From Faulty Electric Energy Meters: Mission Impossible?

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**Learning Overview:** After attending this presentation, attendees will have better insight on several issues and variables that make the reconstruction of electric energy consumption from faulty energy meters a complex and inaccurate task.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting on cases of reconstruction performed by the utility company and shamelessly biased in its favor and by showing how a careful analysis of all available data may yield a more accurate and fairer reconstruction.

Failures and faults on energy meters are quite common, especially in countries, such as Italy, where periodicity of legal verifications on energy meters is set, by law, to every 15 years. Indeed, the most common faults are related more to the current transformers and their connections than to actual faults on the meters themselves.

This presentation covers two cases that led to a partially wrong reconstruction of the energy consumption. The first case is related to an incorrect programming of the Current Transformer (CT) constant inside the meter that measured half of the energy flowing through the metering section. The case was apparently trivial, if not for the case that the incorrect constant masked the fact that the installed power increased to a level that exceeded the CT maximum current, causing an additional error in energy metering. The case could have been set by considering that the supplied customer's production process needed electricity and gas, and the two consumptions were somehow correlated through other relevant process variables, that allowed, by statistical processing, providing a better estimate of the actual consumed energy. Unfortunately, in this case, the implied statistical data processing was too advanced for the judge and the appointed technical expert to understand in the limited time allowed to conclude the technical analysis, and the reconstruction estimated by the utility was considered correct.

The second case is related to an intermittent connection between one phase of the CT and the corresponding terminal of the meter. In this case, a drop in the energy consumption, five years before the inspection that detected the fault, caused by a sudden change in the operations of the customer was considered by the utility company as being caused by the fault, and the customer was charged by a 2M€ bill for the non-metered energy, despite two inspections during this five-year period, that gave evidence that the meter was working correctly, and the fault occurred much closer in time to the last inspection.

Luckily, in this case, the judge and the appointed technical expert understood the reasons opposed by the customer and consequently reduced the utility bill.

By analyzing these two typical cases, this presentation will provide technical experts guidelines on how to consider all available data and sets of information, not only sudden variations in the metered energy values, that may shed a better light on failure inception, how they could affect the measured data, and how to make the consequent reconstruction fairer to the customer.

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**Electric Energy Consumption, Electric Energy Meters, Failures on Electric Energy Meters**

## D15 Home Accessibility Modifications Necessary for a Man With a Cervical Spine Injury

*Robert Dale Lynch, BArch\*, Architectural Expert & Consultant, Haymarket, Prince William County, VA 20169-6203*

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**Learning Overview:** The goals of this presentation are to understand the: (1) consequences of high-level spinal injury; (2) accessibility inspection and analysis of residence; and (3) design of accessibility modifications to residence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing knowledge on how, using data regarding the abilities of an injured and disabled client, to evaluate a residence and execute design modifications to make the residence accessible.

**Background:** The plaintiff was 38 years old at the time of his injury, was married, had two children, and considered himself fit and healthy. He developed pain in the neck and shoulders on November 28 after lifting at work. This failed to settle, and he was assessed by his doctor on December 2 and again on December 5. Physiotherapy was advised.

The plaintiff arranged to see a physiotherapist and during the session on December 7, while lying prone on the table under the therapist's manipulation, he felt an electric sensation going down the spine and into the legs, which then stopped, after which he had lost movement and sensation in both legs. The plaintiff suddenly became quadriplegic, with sensory loss and difficulty breathing. An ambulance was called, he was taken to the hospital, and Magnetic Resonance Imaging (MRI) showed a large, acute spinal disc prolapse at the C6/7 level with acute compression of the spinal cord. The plaintiff remains severely disabled, and will not recover the ability to walk effectively, nor regain bowel bladder or sexual function. He will be wheelchair-dependent permanently.

At the time of injury, the plaintiff's disability was complete loss of power in both legs and loss of sensation from the legs, trunk, and into the inner aspect of the arms. The plaintiff has undergone surgery and intensive, multidisciplinary rehabilitation as an inpatient at a rehabilitation facility. He has regained some sensation, though it remains abnormal. He has recovered some motor function, though still has severe weakness in both legs and remains permanently wheelchair dependent.

**Process:** (1) Review case documents from plaintiff's attorney to determine nature of plaintiff's spinal injuries and his consequent abilities; (2) discover that plaintiff's family currently lives in a geographic location that is conveniently central to the locations of plaintiff's several worksites and proximate to a private parochial school that is providing a good and thorough education for the children, a girl, aged 9, and boy, aged 7; (3) inspect, measure, photograph, and evaluate residence according to the man's accessibility needs; (4) accessibility analysis of residence proves that it cannot feasibly be modified; (5) undertake a search for an alternative home that can be modified to meet the accessibility, usability, and safety needs of the plaintiff and his family. Two are found in the region. One is too distant from the children's school; (6) accessibility analysis of alternate residence proves that it can feasibly be modified; and (7) design of accessibility modifications throughout alternate residence.

**Design of Modifications:** Photographs and drawings of floor plans of residence before and after modifications will be shown.

**Summary of Findings and Recommendations:** The existing residence cannot feasibly be modified to make it safe, accessible, and usable for the plaintiff, considering the consequences of his injury in December. Therefore, an alternative home site had to be found within a reasonable distance of the children's existing school that would be feasibly modifiable to the man's needs and would be conveniently located for him to travel to his several worksites, given the evidence that he is likely to continue to work, albeit on a part-time basis.

The alternate residence found that is available and located about six miles west of the plaintiff's home has been analyzed and found to be feasible to modify.

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### **Injury, Accessibility, Modifications**

## D16 The Biomechanics of Head and Brain Injury

*John D. Lloyd, PhD\*, San Antonio, FL 33576*

**Learning Overview:** After attending this presentation, attendees will better understand the uniquely different forces associated with linear acceleration and those associated with rotational/angular acceleration, along with their effects on head and brain injury, which will assist in the identification of potential mechanical causes of trauma.

**Impact on the Forensic Science Community:** The mechanisms that cause focal and diffuse injuries to the head and brain are unique, with an absence of correlation between the two. This presentation will impact the forensic science community by increasing understanding of these mechanisms to assist forensic investigators in identifying potential mechanical causes of trauma at the scene of the incident.

There are two primary mechanisms associated with traumatic head and brain injury—impact loading and impulse loading. A direct blow transmitted primarily through the center of mass of the head produces impact loading, which can result in extracranial focal injuries such as contusions, lacerations, and external hematomas, as well as skull fractures and underlying brain contusions due to coup and contrecoup brain motions. Rotational movement of the brain relative to the skull induces impulse or inertial loading, which can cause concussion. Inertial loading at the surface of the brain can cause subdural hemorrhage due to bridging vein rupture, whereas its effects on the neural structures deeper within the brain can produce axonal (nerve) injury.

Research conducted in the 1940s first cited angular (rotational) acceleration as the principal mechanism in brain injury. The importance of rotational acceleration in brain injury causation was further investigated in studies involving live primates and physical models, concluding that angular kinematics contributes more than linear kinematics to the generation of concussive injuries, subdural hematomas, and diffuse axonal injuries.

The lab has conducted extensive research on unprotected and helmeted head impacts on a variety of surfaces for the purpose of characterizing head and brain impact features. An array of tri-axial linear accelerometers and tri-axial angular rate sensor installed at the center of mass of a Hybrid III headform is used to quantify the impact. Data from the analog sensors were acquired in accordance with Society of Automotive Engineers (SAE) J211, using a National Instruments® (NI) compact DAQ data acquisition system and LabVIEW™. The raw data was then filtered in MATLAB® using a phaseless eighth-order Butterworth filter with cutoff frequencies of 1,650Hz and 300Hz for the linear accelerometers and angular rate sensors, respectively. An analysis method validated by Takhounts allows for use of an Anthropomorphic Test Device (ATD) to establish a kinematically based Brain Injury Criterion (BrIC) for various types of diffuse brain injury. This method was utilized to express risk of brain injury according to the revised Abbreviated Injury Scale (AIS) scale in terms of peak angular head kinematics.

Results show that the head experiences a primary impact, quantified in terms of peak linear acceleration. Interestingly, impact-related angular velocity of the brain is defined into two peaks: an initial, generally smaller peak that occurs almost concurrent with the peak linear acceleration, followed by a larger induced angular velocity peak. Angular acceleration is divided into three components—direct, induced, and rebound—the magnitude of which is related to the gradient and magnitude of angular velocity. These induced and rebound responses have been overlooked in prior research, but explain why seemingly innocuous impacts can cause devastating effects.

These metrics are quite important in understanding the mechanisms of head and brain injury. An unprotected head impacted by or on a hard surface will likely produce high linear and angular accelerations. Since the duration of impact is typically very short, the effects tend to be more focal, including skull fractures and brain contusions. Whereas impact on or with a softer surface, or involving a helmet, will mitigate some of the linear acceleration by increasing impact time, reducing the likelihood of focal head and brain injuries, but moderate angular kinematics acting on the brain over a longer duration can produce devastating neurological effects, including traumatic axonal injury, hemorrhages, and fatality.

The information and new discoveries to be shared in this presentation will educate forensic investigators as to the uniquely different forces associated with linear acceleration and those associated with rotational/angular acceleration, along with their effects on head and brain injury, which will assist in the identification of potential mechanical causes of trauma.

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### Biomechanics, Head Injury, Brain Injury

## D17 The Curious Case of Asbestos Disease Among Dental Technicians

James Millette, PhD\*, Lilburn, GA 30047-7002

**Learning Overview:** The goal of this presentation is to inform the forensic science community as to how studies can be designed to use forensic microscopical analysis to determine historical exposures to asbestos related to dental technicians.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of the considerations necessary when planning and executing tests to determine historical exposures to hazardous particulates, such as asbestos.

In 1976, after a review of death certificates, Menck and Henderson concluded in their *Journal of Occupational Medicine* article that “Occupational groups found to be at excess risk (of asbestos disease) who have not been previously implicated included roofers, dental technicians ...”.<sup>1</sup> An explanation for the rather surprising entry of “dental technicians” among those with excess risk of asbestos disease was provided in that same year in a report that the Councils on Dental Therapeutics and on Dental Materials and Devices published in the *Journal of the American Dental Association*.<sup>2</sup> It noted that asbestos had been used in dental ring lining tape. From the 1930s until sometime in the 1980s, the inner surface of a crucible or ring used in the “lost wax method” of casting dental prostheses was lined with the asbestos tape prior to casting at a high temperature. In 1980, dentists at the Army Institute of Dental Research in Washington reported on their use of a Scanning Electron Microscopy (SEM) to find that asbestos fibers are released when ring liner was torn from a roll of asbestos by laboratory workers but did not determine the amounts.<sup>3</sup>

To investigate the potential asbestos exposure from dental tape, forensic and occupational microscopy testing was done with Polarized Light Microscopy (PLM), Phase Contrast Microscopy (PCM), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). Chrysotile asbestos contents ranging from 40% to 95% were found in two popular brands of dental tape. Small levels of tremolite asbestos were also found in the tapes. Glovebox testing based on Consumer Product Safety Commission and the United States Environmental Protection Agency protocols was used to determine the levels of asbestos released during the tearing of dental tape. The glove box testing was done in a NuAire 401 sealed glove box (365 liter volume). The released particles were collected on standard air filter cassettes (0.8µm Master Water Conditioning® [MCA] filters) at flow rates between 1 and 5 liters per minute. The filters were examined for fibers using PCM National Institute for Occupational Safety and Health (NIOSH) 7400 procedure and TEM NIOSH 7402.

The glove box tests showed levels ranged from 0.5–4 Fibers/cc. Full chamber tests were done with a protected individual in a controlled room. The test chamber work area was approximately 9ft high by 10ft wide by 12ft long. The study area had a High Efficiency Particulate Absolute (HEPA) air filtration device that was used to clean the area of particulate, including asbestos, before the testing activities began. The HEPA unit ran at a low flow-rate (approximately 45 cubic feet per minute) during the study. Air samples collected and analyzed as described for the glove box studies showed levels of 0.66–4.6 Fibers/cc. Additional glove box tests showed that over 120,000 asbestos fibers (>5 micrometers long) were released during a pair of tears.

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2. Council on Dental Therapeutics and Council on Dental Materials and Devices. Hazards of Asbestos in Dentistry. Reports of Councils and Bureaus. *J. American Dental Association*, 92 (1976): 777-778.
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### Microscopy, Asbestos, Dental Tape

## D18 Roadside Tire Mark: A Useful Source of Supplementary Evidence

*Zygmunt M. Gorski, BA\*, London, ON N5W 1G5, CANADA*

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**Learning Overview:** The goal of this presentation is to educate attendees about the characteristics and meaning of tire marks on roadsides that, in specific instances, can provide supplementary evidence to improve the quality of an investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the fact that the identification and interpretation of roadside tire marks can provide additional, useful evidence in some types of investigations.

Tire marks have been previously studied for identification of specific vehicles in hit-and-run incidents or in interpreting road surface marks that lead to calculations of vehicle speed or vehicle motion. Less focus has been drawn to the useful information that can be obtained from studying tire marks left on roadsides on soft surfaces such as gravel, sand, or a combination thereof, typically found on roadway shoulders. Vehicular travel on hard pavement is difficult to detect unless there is substantial frictional force that causes visible markings. Even tire marks on snow-covered, hard-paved surfaces can be quickly obliterated by passing traffic.

In contrast, tire imprints on softer shoulders are more easily created and less often destroyed. The quality and visibility of these markings are affected by time, environmental conditions, and the characteristics of the soft surface itself. Their presence will also vary with respect to the type of roadway involved and the likelihood that vehicles egress onto a roadside for select reasons. Identification and interpretation of these markings can be useful in instances such as police traffic stops, which may become significant where witness information needs to be compared to the physical evidence, where vehicular loss-of-control needs to be identified, and where roadway safety deficiencies need to be detected.

This presentation will discuss characteristics of tire marks caused by vehicles in positive and negative acceleration. Differences in tire marks caused by changes in direction will also be discussed. The angle of departure of vehicles obtained from tire marks can be related to differences in driver behavior such as loss of control, medical episodes, and falling asleep. Differences in tire marks will be discussed in terms of front and rear wheel drive vehicles as well as overlapping tire marks as related to differences in time/date of occurrence.

Roadway maintenance procedures such as re-grading of shoulders will be discussed as these play a large role in the softness of the surface and whether tire marks are likely to be created. Re-grading causes the removal of any previous markings and an investigator who is familiar with the characteristics of a freshly re-graded shoulder will also become aware that almost all vehicle motions will produce well-defined impressions. Alternatively, shoulders experiencing long durations of dry conditions combined with a clay-like composition may allow few opportunities for leaving identifiable markings.

This presentation will focus on tire marks that were created by naturalistic driving on public roadways with less focus on marks created from controlled testing. While controlled testing may be useful in highlighting specific characteristics of tire marks, the investigator will encounter roadside tire marks in less than ideal conditions where various influences may be at play making identification and interpretation more difficult. There is some benefit gained in providing the investigator with such real-life scenarios.

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### Roadside Tire Marks, Interpretation of Tire Marks, Tire Marks

## D19 Why Not Jail for National Highway Traffic Safety Administration (NHTSA) Crimes: Is Agency Capture to Blame?

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**Learning Overview:** After attending this presentation, attendees will have a new perspective on NHTSA ineffectiveness, prosecutorial discretion, and enforcement methods. This research utilizes the methodology of counterfactual examples of instances that should have been prosecuted and investigates the circumstances which warranted NHTSA's action but, most importantly, identifies the agency's awareness of a crime and exercises its discretion to not act. The theorization of this research, explaining the environment that NHTSA has allowed crime to flourish in the automotive industry and manufacturer-agency convergence to benefit the manufacturers, is agency capture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting crucial new consideration to the law and order aspect of science and safety. The governing bodies that are relied on to enforce standards of safety are a persisting concern of the scientific community. Agency capture is always ripe for consideration. The impact of this research will permeate fields of forensics, policy, enforcement, and public health and safety, plus allow discussion and reopening of old conclusions.

To continue from the introductory research in "EPA and NHTSA Prosecutorial Discretion: A Dichotomy of Effectiveness," this research dissects the reason for sparse enforcement of crimes under the National Highway Traffic Safety Administration. NHTSA maintains a long list of civil settlement penalty cases. Digging into case details, one will encounter an array of phrases like "failure to timely file defect information reports; failure to notify NHTSA of a defect; and failure to report certain death and injury incidents." These actions by auto manufacturers, which were pursued only as civil penalties, are directly chargeable as criminal offenses with imprisonment under 49 U.S.C. § 30170 and Title 18. There are zero records of an auto manufacturer incarcerated for crimes under NHTSA jurisdiction. Prior theories suggest a lack of funding and manpower to investigate every defect report and consumer complaint as the issue, but NHTSA's defect reporting program was designed in favor of manufacturers, lobbied by automakers from the start. Defects are investigated on a basis of "extraordinarily more defective than other defects." This regulatory program has been a useful scapegoat for NHTSA not to investigate a manufacturer, despite the historical voluminous number of "crimort" defect lawsuits in lower courts that NHTSA were (or should have been) notified of by law via the TREAD Act, providing evidence to warrant investigation.

In prior research asking "Why not jail for auto executives?," frequent themes identify problematic rule structures, insufficient staff, budgetary shortcomings, deferred prosecution agreements, criminal vs. civil penalties, lack of individual accountability, and lobbying at the rulemaking stage as the culprit for the lack of criminal cases. While the foregoing issues erode the integrity of motor vehicle safety, preexisting theories negate a significant possibility—agency capture. Using the following case studies, data indicative of agency capture persist: the Federal Motor Vehicle Safety Standard 207 insufficiency and 15-year cover-up by GM expert and NHTSA complacency; and intervention of NHTSA Administrator, Jaqueline Glassman, a former Chrysler lawyer, to stop Federal Motor Vehicle Safety Standards (FMVSS) 207 rulemaking action during the *Flax v. Chrysler* trial in Federal court in November 2004; wherein, Chrysler withheld prior findings of FMVSS 207 defects and prior Chrysler expert testimony proving known dangers of defective seats and seat belts.<sup>1</sup>

This research theorizes that NHTSA became aware of criminal incidents or defects, but some degree of responsible action was not taken by the agency, and there are relationships converged between NHTSA employees and auto manufacturers, indicative of agency capture resulting in ineffective prosecution. For the purposes of this theory, this study maintains the Bagley definition of agency capture: "The phenomenon whereby regulated entities wield their superior organizational capabilities to secure favorable agency outcomes at the expense of the diffuse public."<sup>2</sup> The financial incentives for avoiding defect recalls are immense. However, there is a far greater issue when root to tip there is an industry stronghold on the agency. From lobbying in rulemaking of material standards to save pennies on the dollar, avoiding multibillion dollar recalls by "playing ignorant," to ensuring that no true consequence will be paid for the crime. The environment in which criminal activity and unsafe vehicles flourish in the automotive industry has been bred and born from NHTSA's turn-cheek or collaborative disposition. The degree to which capture has eroded the agency is exhibited in the decades of continued faulty safety standards, frequent defects, and execrable resultant death and injury tolls.

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### Agency Capture, Crimort, NHTSA Crime

## D20 Evaluating the Effect of the Crash Locking Tongue on the Breaking Strength of Seat Belt Webbing

*Kurt D. Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA 93190*

**Learning Overview:** The goal of this presentation is to demonstrate through rigorous laboratory testing the effect of a cinch-type latch plate on reducing the breaking strength of automotive seat belt webbing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing test results obtained through rigorous laboratory testing that clearly demonstrates the failure mode and load of a cinch-type latch plate.

On January 10, 2018, the National Highway Traffic Safety Administration's (NHTSA) Office of Defects Investigation (ODI) began investigating two incidents of seat belt separation. The separations occurred in two New Car Assessment Program (NCAP) tests conducted on the 2018 Volkswagen® (VW) Tiguan®, the first year of a new vehicle platform. The NCAP test is a 35mph, full frontal, rigid barrier crash with a belted 50<sup>th</sup> percentile male Anthropomorphic Test Device (ATD) driver and a belted 5<sup>th</sup> percentile female ATD front passenger. The tests were conducted on December 8, 2017, at MGA in Wisconsin, and December 13, 2017, at TRC of Ohio. The test reports are not currently available on NHTSA's website. During the crash tests, the driver seat belt webbing completely separated in tension at the latch plate, a device that VW calls a Crash Locking Tongue (CLT) (Figure 1). The CLT is a device that switches under load and the webbing is clamped, resulting in greater loads applied to the lap belt. Approximately 290,000 Tiguan® vehicles use the CLT device.



*Figure 2 - The Crash Locking Tongue of the 2018 Volkswagen Tiguan.*



*Figure 1 - The front outboard seat belt sill-end pretensioner of the 2018 Volkswagen Tiguan.*



*Figure 3 - Three-point anchoring held the webbing with an included angle of approximately 45 degrees.*

On July 3, 2018, ODI upgraded their analysis to further examine the seat belt behavior to determine, in part, a root cause for the seat belt separation. Their research included a review of any vehicle and seat belt design changes for the 2018 Tiguan® platform and their effects on the performance of the seat belt system. Based on its analysis of the NCAP test results and numerous sled tests, VW asserted that the ATD umbilical cord exerted additional forces on the seat belt system, causing the separation. However, a micro analysis conducted by the National Transportation Safety Board (NTSB) verified the seat belts had failed in tension and not due to cuts or abrasion. To date, a safety recall campaign has not been opened to address this potential safety hazard.

Testing was performed on new seat belt assemblies to investigate the effect of the CLT design on reducing the breaking strength of the webbing. The type-2, dual-pretensioner (sill-end and retractor) assembly features 47mm-wide polyester webbing and a dual-locking, switchable load-limiting retractor. The sill-end pretensioner is capable of producing a peak force of approximately 6,228N in the lap belt (Figure 2). Three-point anchoring held the seat belt webbing in a "V" shape with an included angle of approximately 45 degrees, similar to in-vehicle geometry (Figure 3). The end of the lap belt webbing was held using the stitched loop end for the first test and subsequently by a split-drum grip. The lap belt was preloaded to between approximately 3,959N and 4,448N. The latch plate tongue was held by a clevis pin. The end of the shoulder belt webbing was held by a split-drum grip secured to a hydraulic cylinder. The shoulder belt web-grip displacement rate was between approximately 89 and 94mm/s. Data was sampled at 1,000Hz.

Federal Motor Vehicle Safety Standards (FMVSS) 209 S4.4(b)(6) requirements for type-2 assembly performance hardware specifies that any webbing cut by the hardware shall have a breaking strength of not less than 15,569N for the lap belt or not less than 12,455N for the shoulder belt. In this study, separation occurred at the CLT with peak lap belt webbing loads between approximately 6,993N and 8,203N (SD 512N) and peak shoulder belt webbing loads of between approximately 10,280N and 11,521N (SD 519N). Pre-loading the lap belt (which occurs with sill-end pretensioner activation) caused the CLT to clamp the webbing. The clamping edge of the CLT appears to have caused the webbing separation.

The test series demonstrated the CLT design reduced the breaking strength of the seat belt webbing so the assembly no longer passed applicable standards. The reduction in restraint load capacity results in a vulnerability to total loss of seat belt restraint in traffic collisions.

### **Crash Locking Tongue, Webbing Breaking Strength, FMVSS 209 S4.4(b)(6)**





## **D21 Automotive Defect Case Studies Exemplifying Agency Capture of the National Highway Traffic Safety Administration (NHTSA)**

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**Learning Overview:** The goal of this investigation is to explore the poor job that automakers and the Federal government have done regarding their clear obligations in automotive safety. Case histories will provide a clear and accurate representation of the inadequacies and facts regarding several Federal Motor Vehicle Safety Standards (FMVSS), as well as how agency capture has enabled automotive defects to continue unabated for decades, despite ample proof of inexpensive alternative designs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community involved in the forensic aspects of traffic accident investigation and reconstruction.

Several case studies illustrate how widespread, life-threatening vehicle safety defects have been ignored for decades by automakers and the NHTSA. This research illustrates that this is due, at least in part, to agency capture of Department of Transportation (DOT) officials. (1) Petitions to upgrade FMVSS 207 and to establish a rear impact occupant protection standard have been “under review” at NHTSA since 1974. This has occurred despite thousands of preventable deaths, countless catastrophic injuries, and research clearly proving far safer, economically feasible alternative designs. NHTSA admitted in 1996 that FMVSS 207 is “flawed and inadequate” but has yet to change the standard. (2) Roof crush resulting in failure of vehicle survival space during rollover crashes kills approximately 10,000 Americans yearly, about 30% of all light vehicle occupant fatalities. The number of occupant injuries is significantly higher. FMVSS 216, the original standard and subsequent FMVSS 216a, define vehicle roof strength requirements. The results of hundreds of rollover crash tests and accident investigations involving roof crush and the questionable effectiveness of these standards, and the decades of delay involved in upgrading the standards, will be discussed, analyzed, and summarized. (3) Flaws in FMVSS 301 for fuel system integrity has allowed extremely dangerous vehicles to be sold to the motoring public. Even after the debacles of the Ford® Pinto®, General Motors® sidesaddle gas tank pickups, and more recent Jeep® fuel-fed fires, automakers continue to build vehicles with fuel tanks placed in crush zones and to ignore readily available inexpensive technology that would significantly reduce the threat of crash-induced fuel system failures. (4) The Minicars Research Safety Vehicle (RSV) was developed in the 1970s to prove that fuel-efficient, yet extremely safe vehicles were readily producible by automakers. When NHTSA later claimed that it was impossible to have safe fuel-efficient cars, they intentionally destroyed the evidence of the remaining RSVs.

All these areas exemplify how agency capture has occurred in violation of public trust. It will be seen that automakers and the government have, and continue, to fail to provide reasonable protection to the public in the event of automotive rear impacts, rollovers, and crash-induced fires. Automakers continue to provide misleading or untrue information to NHTSA, which in turn continues to turn a blind eye to ongoing serious safety defects.

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### **Automotive Safety Defects, Agency Capture, NHTSA**

## D22 Forensic Differentiation of Ink Samples Using the Pyrolysis-Gas Chromatography/Mass Spectrometry (GC/MS) Technique

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**Learning Overview:** After attending this presentation, attendees will: (1) learn what the pyrolysis-GC/MS technique is and how its different modes of operations (such as Evolved Gas Analysis [EGA], thermal desorption, flash pyrolysis, and heart-cutting) can be applied for material characterization of ink and dye samples; and (2) discover how pyrolysis-GC/MS can be applied to quality control, reformulation, contamination, unknown identification, and failure analyses while increasing laboratory efficiency by eliminating sample pretreatment and the “traditional” solvent-based techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a relatively new and modern-day analytical technique for material characterization using pyrolysis-GC/MS. This technique allows laboratories to eliminate the time-consuming sample preparation and solvent extraction while improving precision and accuracy in analytical protocols.

In forensic investigations, the identification and differentiation of inks and dyes need to be done quickly and accurately, while the analytical method is straightforward. Most of the traditional methodologies for the determination of inks are based on solvent extraction, filtration, and concentration. These techniques are cumbersome, time-consuming, and suffer from analyst-to-analyst variability while producing data of limited value.

Today, many laboratories are integrating modern-day pyrolysis-GC/MS using the micro-furnace pyrolyzer in their analytical protocols due to the efficiency and accuracy of this technique for analyzing the organic composition of virtually any material. This technique consists of the micro-furnace pyrolyzer, which utilizes a low thermal mass ceramic furnace and a GC/MS system. The micro-furnace is directly connected to the GC injection port and enables scientists with multiple analytical techniques in addition to flash pyrolysis. This technology uses heat to thermally extract additives and light compounds from heavier and polymeric materials; any solvent extraction or sample pretreatment can be then eliminated. Due to the accuracy and precision of the micro-furnace temperature control ( $\pm 0.1^\circ\text{C}$ ), this technology is used for quantitative analysis while producing highly reproducible data.

In this presentation, a strategic “chemical method map” approach is described using multiple modes of the micro-furnace pyrolyzer to characterize and differentiate two ink samples. The two black ink samples are analyzed and compared to identify any minor differences in their chemical composition while eliminating any solvent extraction or sample pretreatment.

The first step when developing the “method map” is the Evolved Gas Analysis (EGA) technique. EGA provides a “picture” of the sample’s complexity and thermal profile. In this technique, the sample is dropped into the micro-furnace, which is at a relatively low temperature. The furnace is then programmed to a much higher temperature. Compounds “evolve” continuously from the sample as the temperature increases, then a plot of detector response versus furnace temperature is obtained. The EGA thermogram is very well correlated to Thermal Gravimetric Analysis (TGA) and guides the scientist on what to do next for further separation analysis and characterization. EGA thermogram shows if thermal desorption, flash pyrolysis, or heart-cutting is needed, or if a combination of the techniques is required.

The second step is to analyze the thermal zones of interest obtained from the EGA thermogram. In this step, the selective sampler slices a thermal zone out of the sample and separates the components chromatographically using an MS. This technique is called Heart Cutting (HC).

In this report, using EGA/MS and HC modes of operations of the micro-furnace, the two ink samples, including their raw materials, are chemically characterized. From the analysis, it could be concluded that one of the ink samples contained a much higher concentration of naphthol as the unreacted raw material from the dye ingredient of the ink sample.

### Pyrolysis GC/MS, Forensic Differentiation of Inks, Material Characterization

## D23 Toward a Practical Standard for Quantification of the Edge and Tip Sharpness of Knives and Other Sharp Weapons

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**Learning Overview:** After attending this presentation, attendees will understand how to assess the sharpness of knives and other implements on an objective scale and assess whether or not they would easily penetrate skin in a stabbing or slashing attack.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing pathologists and other forensic science professionals to objectively assess the penetration ability of weapons in a quantitative and repeatable way that changes the assessment from an opinion-based assessment to an objectively based assessment.

Since the publication of the National Academies' Report on *Strengthening Forensic Science in the United States: A Path Forward* in 2009, there has been a strong call internationally for the development of relevant high-quality forensic standards.<sup>1</sup> In addition, many courts examine testimony based on the *Daubert* standard that requires experts to understand the basic theory, any standards pertaining to the experimental technique, the sources and likely magnitudes of error, as well as a number of other criteria aimed at improving the quality of forensic evidence provided to the courts.

Sharp instruments such as knives are commonly used in violent crime, including homicides, street-based and armed robberies, sexual assaults, and terrorism, particularly in those countries where guns are not common. Previous work has shown that forces generated by volunteers stabbing skin simulants and porcine samples with knives and screwdrivers generate a range of results. Men generate approximately twice the force of women. Forces generated by volunteers who were asked to stab with mild, moderate, and severe forces were in almost all cases significantly greater than the force required to penetrate skin for stabbing with sharp knives. The conclusion from this work was that in order to understand a stabbing attack, it is important to understand the tip radius of the weapon, the sex of the assailant, the minimal force for penetration, and whether the force required for penetration is greater than that which can be generated by the assailant.

The work in this presentation is aimed at quantifying the sharpness of the edge and tip of a knife by combining separate industry-standard approaches. This is then related to the ability of the knife to penetrate skin and clothing in a reproducible way that can be readily used by the forensic community.

Edge sharpness was measured for a range of knives using a Brubacher edge sharpness test. This study measured the edge sharpness at several different points on the blade profile, from near the tip to toward the hilt of the blade. This study also performed reproducibility tests to understand the level of scatter in the results.

Tip sharpness was assessed by using a series of rectangular openings and assessing the depth to which the tip penetrates. If the tip penetrates an opening to sufficient depth, an indicator light will illuminate to allow the pathologist or scientist to record the result. A blade tip profile factor is also introduced to generate an assessment of the ability of a knife to penetrate.

The combination of the tests is then collated to allow the pathologist/scientist to assess the implement as being very sharp, sharp, moderate, blunt, or very blunt. This can then be used to quantitatively support the subjective scale they have chosen to use when proposing the minimal force that may have been required for the knife to penetrate the body.

The proposed tests will allow forensic practitioners to develop a common understanding of knife/implement sharpness that will assist the courts in having a consistent approach to understanding the sharpness of knives and other implements used in violent crime without the need for more complex laboratory weapon testing.

### 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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### Sharpness, Knives, Quantification

## D24 The Environmental Protection Agency (EPA) and National Highway Traffic Safety Administration (NHTSA) Prosecutorial Discretion: A Dichotomy of Effectiveness

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**Learning Overview:** The goal of this presentation is to discuss the undeniable dichotomy between the EPA and the NHTSA in their prosecutorial discretion, as exemplified by NHTSA's miniscule criminal prosecution rates of negligent manufacturers and corporate malfeasance, despite death and injury tolls in the thousands.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new perspective on the underlying environment in which crime is allowed to flourish in the automotive industry and enlighten the possibilities of ineffective prosecution by NHTSA, which negatively impacts regulatory compliance and vehicle safety. The forensic science community will benefit from salience of this research when investigating automotive defects by considering the industry-wide disposition toward compliance but also the degree to which NHTSA monitors and enforces such behavior.

By enumerating and highlighting the rigorous Department of Justice (DOJ) criteria that the EPA follows to warrant investigation of crimes, attendees will gain perspective on the small extent to which NHTSA has exercised its statutory power to investigate and charge individuals. The methodological purpose of comparing the two agencies is not only their underlying mission of protecting public health and safety and their similarities in permitting and documentation protocol, but also their jurisdictional cross-overs in policy, making the effectiveness of both agencies a concern to political and safety sciences in both disciplines.

To continue from previous research, "Criminal Engineering Without Consequence, Why?", the longstanding automotive industry behavior of violating safety standards, ignoring imminent defects, and falsifying scientific discovery has led to decades of dead and severely injured passengers.<sup>1</sup> The industry excuse for such behavior has been for budgetary bonuses and cost cutting in manufacturing materials, at the expense of human safety. The means to which NHTSA fights against such atrocities has been limited to defect reporting, with criminal sanctions available under 49 U.S.C. § 30170 and Title 18. However, unique patterns have presented themselves in the comparative analysis between NHTSA and EPA regarding the number of crimes prosecuted under respective agencies.

This research asserts that the massive difference in criminally prosecuted cases between the EPA and NHTSA is not due to EPA overzealousness, but rather NHTSA ineffectiveness. The EPA has an extensive database of federally criminally prosecuted cases, including those resulting in prison time for individuals. Meanwhile, NHTSA holds a long list of civil penalties but have yet to criminally charge and imprison an individual under 49 U.S.C. § 30170 or Title 18 for failure to report a defect. Only the small handful of high-profile defect scandals such as Toyota®, General Motors®, and Takata®, once they reached stifling publicity, have resulted in criminal charges against individuals and executives—yet they resulted in Deferred Prosecution Agreements (DPAs). To date, there is no known automotive executive that has served jail time pursuant of charges by NHTSA. Meanwhile, the methods that NHTSA utilizes to monitor and investigate defects relies on consumer complaint reports and manufacturer good faith and integrity to notify the agency of a potential problem. There is a gross disparity between the number of known automotive defects that caused death or catastrophic injury and the number of criminally charged manufacturers or their responsible officers. Though research supports preexisting literature regarding the corrosivity of DPAs in their inability to deter crime and support the mission of the agency, it primarily concludes with the troubling question: what of the crimes that were never investigated or charged, let alone received a DPA? This research lays the foundation for an even greater in-depth analysis, as shown in a follow-up presentation, of NHTSA effectiveness, prosecutorial discretion, and enforcement methods with the resultant theory of agency capture.

### Reference(s):

<sup>1</sup>. *Proceedings of the American Academy of Forensic Sciences, 72<sup>nd</sup> Annual Scientific Meeting, Anaheim, CA. 2020. D33.*

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### Ineffective Prosecution, Automotive Safety Defects, EPA vs. NHTSA

## D25 The Role of Standards and Case Law Relating to Forensic Analysis in Walkway-Safety Incidents: Part One

Mark I. Marpet, PhD, PE\*, MMA, Inc, Chester, NJ 07930

**Learning Overview:** The goal of this presentation is to discuss the respective roles of codes, standards, professional practice, and judicial precedent with respect to the question of what constitutes an actionable defect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware of the relationship between engineering and safety-professional standards and upon case law and, based upon that, whether an incident is actionable or not.

When an ambulation-related accident occurs, it is important to determine whether the accident was caused by a failure of or defect in the premises' design, construction, or maintenance. If yes, the question then becomes whether or not that failure or defect is actionable. The rubric is that if an element is related to design or construction, for that element to be a failure or defect, it must have been recognized as such at the time the element was designed or constructed. If the element is related to maintenance, an ongoing process, the element under study had to have been recognized as problematic at the time of the accident.

A building, unless it had been erected in pre-code times (the late 19<sup>th</sup> century in some large cities, as late as the mid-20<sup>th</sup> century—or not at all—in some rural areas), would have to conform to the building code in effect at the start of construction. (The examples in this paper refer to the New York City Building Codes.) For example, a building erected in New York City in the 1950s had to be in conformance with the 1938 New York City Building Code (the “Code”). That building also has to conform to any property maintenance codes in effect at the time of an accident. (Property maintenance codes generally mimic common law [i.e., property owners must keep premises in “safe condition”]. Thus, they add little of substance (and much to the imagination) of forensic practitioners.) Beyond the building and property-maintenance codes, there exists a host of other standards, typically non-mandatory, either promulgated by companies having an interest in a specific topic or voluntary-consensus standards, promulgated by interested parties through a Standards Development Organization. Beyond that, there is the question of what constitutes Acceptable Practice at the times of design, construction, and ongoing maintenance. All of this is filtered through the lens of litigation, in which courts receive into evidence engineering and safety practice in the form of “expert opinion” that serves as a guide, but not a mandate.

The need to look beyond specific standards is aptly illustrated by two simple—but not simple—examples relating to stair handrails: the height of a handrail above a flight of stairs, immediately below, and the requirement for handrails at building entrances, in Part Two.

**Example 1:** Handrail height. Handrails were not mentioned at all when the Code was first enacted in 1899; they are mentioned only in passing in the State of New York Tenement–House Act, which came into effect in the first decade of the 20<sup>th</sup> century, to wit: in every tenement house all stairways shall be provided with proper banisters and railings and kept in good repair. The 1916 Code provides that stairs “shall have hand-rail on both sides.” The 1938 Code further specified that handrails must be placed 30–34 inches above the tread nosing. The 2008 Code revised the height requirement to 34–38 inches above the tread nosing. These changes over time generate interesting questions: (1) If a building had been erected in 1955 and the handrail height was measured to be 36 inches above the stair nosing, can it be considered defective? It surely violates the code in effect when the building was erected, but it meets the current handrail requirement; and (2) The inverse situation is also problematic. Consider a building built in 2015 having handrail heights of 32 inches above the step nosing. Is that handrail defective? Consider that thousands of buildings built before 2008 were required to have handrails between 30–34 inches.

Ultimately, the analysis should focus upon the relationship between handrail height and stair-user safety, starting with the basis for the Code revision. However, research relating to the higher handrail height is incomplete. Maki discussed a partial answer in researching handrail grasping from a static position, but the kinematics of grasping for and holding onto a handrail was not researched.<sup>1,2</sup>

Short of the existence of definitive evidence that the 2008 Code change either eliminated or (heaven forbid) substantially increased staircase accidents in structures; a forensic practitioner should have an uphill battle arguing that any handrail height between 30–38 inches could be unsafe or accident causal based solely upon reliance of one of the two editions of the Code.

This is a simple example: a single measurement and a single requirement. Yet it can stir a hornet’s nest of disagreement.

### Reference(s):

1. Maki, B.E., Bartlett, S.A., Fernie, G.R. Influence of stairway handrail height on the ability to generate stabilizing forces and moments. *Human Factors*, 1984
2. Maki, B.E., Bartlett, S.A., Fernie, G.R. Effect of Stairway Pitch on Optimal Handrail Height.” *Human Factors*, 1985.

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### Standards, Codes, Case Law

## D26 The Role of Standards and Case Law Relating to Forensic Analysis in Walkway-Safety Incidents: Part Two

Mark I. Marpet, PhD, PE\*, MMA, Inc, Chester, NJ 07930

**Learning Overview:** The goal of this presentation is to discuss the respective roles of codes, standards, professional practice and judicial precedent with respect to the question of what constitutes an actionable defect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the relationship between engineering and safety-professional standards and case law and, based upon that, whether an incident is actionable.

Beyond the complexity posed by revisions of standards, which reflect both modern building practice and an evolving understanding with respect to building safer structures, a profound disconnect exists between what forensic practitioners argue, based upon accepted engineering and safety practice, and the actual outcome of a particular lawsuit. In court, statutes, codes, and case law are dispositive, not expert opinions, which only serve to inform the former. That is, and safety professionals may not like this at all, courts set the rules for what is acceptable societal risk, not engineers or safety professionals. Courts can, and generally do, include forensic analysis in their decision-making, but do not uncritically adopt it. In general, the legal system engages in a more nuanced analysis of the totality of the unique facts presented than does a strictly standards-based analysis. To understand this, one must look at the structure and application of standards vis à vis legal precedent. Standards and regulations take various forms. From least to most authoritative, standards and regulations form a hierarchy: (1) company standards, (2) industry-wide standards, (3) voluntary-consensus standards, and (4) government-generated rules and regulations. Let's briefly look at each. (1) Company standards are just that: a set of rules and specifications, akin to instructions on how to properly use a given product. For example, a company standard could instruct an installer how to properly install a specific model of handrail on a given type of wall. (2) Industry standards tend to be far less granular than company standards. Industry standards are generally developed by the members of an industry group. An industry standard might require that a handrail be installed in a manner strong enough to perform its function at a code-specified height. Industry standards are written to cover a class of product, rather than a specific brand and model. (3) Voluntary-consensus standards are like industry standards except that the members of the standards-development committee are drawn from all interested parties and, with few exceptions, are "refereed" by a Standards Development Organization (e.g., American Society for Testing and Materials [ASTM] International [formerly the American Society for Testing and Materials], the American National Standards Institute [ANSI], and the American Academy of Forensic Sciences Standards Board [ASB]). Standards generated by such groups must take into account the often-divergent, sometimes conflicting views and interests of the various stakeholders. In this example, handrail height may have to be adjusted in an elementary-school setting. (4) Government-generated rules and regulations. These are usually quite generic, as they have to be generally applicable. They often rely upon standards developed by others. In our example, the handrail height is set by Code at 30–34 inches before 2008 and 34–38 inches thereafter.

**Example:** The requirement for handrails at building-entrance steps. Case law at first blush can seem arbitrary or counterintuitive. For example, the New York City Building Code has, since 1916, required handrails for both exterior stairs and interior stairs.<sup>1</sup> But one case, *Gaston v. NYCHA* extensively parsed out the specific language of the code sections at issue to hold that stairs on the outside of a building that lead to the front door do not need handrails. The court found that if exterior steps lack specific features (e.g., a roof, like the covered stairway attached to a wall outside a movie theater), they are not considered "required" stairs and do not have to meet Code.

Thus, a forensic practitioner would find, at least in Manhattan and the Bronx, that the sensible argument that stairs from the building entrance to the street needs handrails that can be reached by all using the stairs would hold no sway. From an engineering or safety point of view, that result would seem to be irrational: one cannot get from the front door to the street without going down steps, seeming to make the steps at issue quite literally necessary, but not "required." However, from a legal point of view, they are not "required," and—again—statutory analysis trumps safety analysis.

The reality of New York City architecture is that it is common for Manhattan buildings, especially older buildings, to not have handrails at entrance stairs and stoops. The *Gaston* result reflects the reality of city's building entrances.

The takeaway is that it is not engineers and safety professionals, it is society, through the legislature and, ultimately, the courts, that set society's level of acceptable risk. Clearly, safety professionals point out that buildings would be safer if they all had proper height handrails on all stairs. But society, through the case law, says that buildings can be safe enough without them.

### Reference(s):

<sup>1</sup>. §154 in the 1916 code, §6.4.1.10 in the 1938 code, §27–376 in the 1968 code, and §BC 1022 in the 2008 code).

### Standards, Codes, Case Law

## D27 Partitioning Relationships to Quantitate Organic Molecules in Air or Breath Samples

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**Learning Overview:** After attending this presentation, attendees will have learned about methods to quantitate organic molecules in air or breath samples, with a focus on sorbent-air and blood-air partition coefficients.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing methods to quantitate organic molecules in air or breath samples through the use of sorbent-air and blood-air partition coefficients. Quantitative partitioning information is crucial to numerous areas of forensic science; however, this presentation will focus on its application to the development of a cannabis breathalyzer device.

There is interest in terpenes and terpenoids produced by cannabis plants, which may be found in indoor environments such as greenhouses, isolated plant material, or exhaled breath. Cannabis plant material can be distinguished from similar plants by its major cannabinoids ( $\Delta$ -9-tetrahydrocannabinol or cannabidiol); however, cannabinoids have low vapor pressures and are reactive; only small quantities will be captured at ambient temperatures.<sup>1</sup> Furthermore, highly odorous compounds that may be important for cannabis detection by humans or trained canines are not necessarily the compounds present in the highest concentration in the vapor phase.<sup>2</sup> Recent investigations indicated that three sesquiterpenes ( $\alpha$ -santalene, valencene, and  $\beta$ -bisabolene) are unique to cannabis, suggesting that terpenoids may be effective markers for cannabis for some applications.<sup>3</sup>

When air or breath equilibrates with a sorbent, sorbent-air partition coefficients describe the relative concentrations of molecules in the two phases and are required to quantify the original concentration of the analyte(s) of interest. For breath samples, blood-breath partition coefficients may enable blood concentration to be calculated from breath concentration. Passive air samplers are frequently employed to characterize exposure to hazardous chemicals in indoor and outdoor environments with adsorbent configurations designed to sample in the kinetic region (linear uptake) or the thermodynamic region (equilibrium).<sup>4</sup> Multiple adsorbents can be used, including activated charcoal and Polydimethylsiloxane (PDMS), which is deployed in the form of sheets or as sorbent-coated glass fibers. The exhaled breath of cigarette smokers and non-smokers was recently sampled with a capillary packed with sorbent-coated glass microfibers, resulting in high surface area that promoted sorbent-breath equilibration.<sup>5</sup>

This presentation describes research on PDMS, which can be coated onto glass fibers and has been used to concentrate analytes from both headspace and breath samples. Quantitative analyses require sorbent-air partition coefficients at both adsorption and desorption temperatures, which span at least 20°C to 200°C. Partition coefficients can be extrapolated within the linear range of the van't Hoff equation if sufficient experimental values are available; often they are not. This presentation will describe an empirical, temperature-explicit group contribution model to predict sorbent-air partition coefficients solely from molecular structure.<sup>6</sup> This study created the model with training compounds containing carbon, hydrogen, and oxygen, reflecting interest in phytochemicals such as terpenoids. Partition coefficients for the training compounds were calculated from Kovats retention indices and isothermal gas chromatography measurements at temperatures from 60°C to 200°C. This study compared model predictions to limited experimental values available at 25°C. The value of this modeling approach is its ability to incorporate all available data, which is advantageous for properties with limited experimental values at a single temperature. This presentation also discusses the important role of blood-air partition coefficients and investigates the use of static and dynamic headspace sampling methods for their measurement.

### References:

1. Tara M. Lovestead and Thomas J. Bruno. Determination of Cannabinoid Vapor Pressures to Aid in Vapor Phase Detection of Intoxication. *Forensic Chemistry* 5 (2017) 79-85.
2. Somchai Rice and Jacek A. Koziel. The Relationship Between Concentration and Odor Activity Value Explains the Inconsistency in Making a Comprehensive Surrogate Scent Training Tool Representative of Illicit Drugs. *Forensic Science International* 257 (2015) 257-270.
3. Nancy Wiebelhaus, D'Nisha Hamblin, Natasha M. Kreitals, and Jose R. Almirall. 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* 2 (2016) 1-8.
4. Aaron Lamplugh, Megan E. Harries, Feng Xiang, Janice Trinh, Arsineh Hecobian, Lupita D. Montoya. Occupational Exposure to Volatile Organic Compounds and Health Risks in Colorado Nail Salons. *Environmental Pollution* 249 (2019) 518-526.
5. D'Nisha Hamblin and Jose R. Almirall. Analysis of Exhaled Breath from Cigarette Smokers using CMV-GC/MS. *Forensic Chemistry* 4 (2017) 67-74.
6. Kavita M. Jeerage and Elijah N. Holland. Predicting Sorbent-Air Partition Coefficients for Terpenoids at Multiple Temperatures. *Industrial & Engineering Chemistry Research* 59 (2020) 16473-16482.

### Breath Analysis, Partition Coefficient, Group Contribution Model

## **D28 An Overview of an Integrated Cannabis Measurement Services Program to Help the Forensic Community in the Determination of $\Delta$ -9-Tetrahydrocannabinol ( $\Delta$ -9-THC), Tetrahydrocannabinolic Acid (THCA), and Total THC in Seized Cannabis (Hemp and Marijuana)**

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**Learning Overview:** The goal of this presentation is to provide forensic scientists with an overview of a recently developed cannabis research program that is developing analytical tools to help in confidently distinguishing seized cannabis samples as marijuana or hemp.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by summarizing new analytical methods, a cannabis Quality Assurance Program (QAP), and hemp reference materials underway to help forensic laboratories to confidently distinguish seized cannabis samples as marijuana or hemp.

Cannabis (marijuana and hemp) and its psychoactive constituent,  $\Delta$ -9-THC, have been classified as Schedule I controlled substances since the 1970s. In the past, seized cannabis samples have been tested by forensic laboratories, who verify the identity of the plant through macro- and microscopic evaluation. The presence of  $\Delta$ -9-THC through presumptive (colorimetric) and confirmatory chemical testing via Gas Chromatography/Mass Spectrometry (GC/MS). Currently, marijuana and  $\Delta$ -9-THC remain on the controlled substances list, although medical marijuana is legal in 33 states and recreational marijuana is legal in 11 states as well as the District of Columbia. The 2018 Farm Bill defined hemp as cannabis containing less than or equal to 0.3 % potential  $\Delta$ -9-THC content and removed hemp from the controlled substances list. These legal changes have required forensic laboratories throughout the United States to implement quantitative analytical methods to distinguish cannabis seizures as marijuana or hemp. However, the majority of these laboratories have little to no experience in or accreditation to perform quantitative drug analysis.

To help federal, state, and local forensic laboratories, a new integrated measurement services program has been developed to provide analytical tools to confidently distinguish seized cannabis samples as illegal marijuana or legal hemp. The new cannabis program will help ensure the quality of routine analysis in forensic laboratories with a three-pronged approach: (1) robust analytical methods for a range of techniques on a variety instrumental platform; (2) cannabis Reference Materials (RMs); and (3) cannabis Quality Assurance Program (QAP). This presentation will provide a summary of these new developed analytical tools that permit the accurate measurements of  $\Delta$ -9-THC, THCA, and total THC in cannabis plant, oils, and edibles samples. The analytical techniques being developed include GC/MS, Liquid Chromatography with Ultraviolet Detection (LC-UV), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), Near-Infrared (NIR), and sample extraction protocols, which will be used for the characterization of all potential cannabis RM and QAP samples. RMs are a critical measurement service that is presently lacking and could provide forensic laboratories with quality control materials to be used as part of their validation schemes. In addition, a cannabis QAP will help forensic laboratories demonstrate and improve measurement comparability and/or competence. Participation in a cannabis QAP will also help assist in the design and characterization of cannabis RMs. The first exercise of a cannabis QAP is focusing on the determination of cannabinoids in two different hemp oils. In addition, attendees will also be asked to identify the type of sample preparation and analytical methods employed in their testing to facilitate conclusions about potential method bias.

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### **Seized Samples, Cannabis, $\Delta$ -9-THC**



## D29 A Quantitative Assessment of Last Words Using Suicide Note Assessment REsearch (SNARE)

Cristina Aggazzotti, PhD\*, Meridian, ID 83642

**Learning Overview:** After attending this presentation, attendees will have learned a method of quantitatively assessing the last words of offenders on death row and will better understand the similarities and differences between last words and other related text genres, such as suicide notes and apology letters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing computational methods in forensic science in two primary ways: (1) by testing the capabilities of one such quantitative method, which has previously only been used to identify suicide notes and suicidal ideation, to capture a different text type—the last words of inmates on death row; and (2) by quantitatively determining how similar last words are to suicide notes and other relevant genres, such as apology letters, love letters, and trauma narratives.

Many previous studies on the last words of inmates on death row used qualitative approaches, such as thematic analysis. However, studies have increasingly begun incorporating quantitative methods to examine common themes, and emotional language use.<sup>1,2</sup> Some of these studies have compared last words to suicide notes, finding that both text types share many features (e.g., themes of love and regret), but also differ in clear ways (e.g., lower rates of cognitive constriction in last words (32%) compared with that in suicide notes (87%)).<sup>1,3</sup>

One current quantitative method for identifying and classifying suicide notes is SNARE. SNARE is a tool that uses linear discriminant function analysis with leave-one-out cross-validation to compare a questioned document, such as an apparent suicide note, to a database comprising >400 real suicide notes and 500 control documents across a range of text types, including apology letters, love letters, trauma narratives, angry letters, complaint letters, simulated and real threats to known targets, business letters related to insurance, and a small number of simulated and hoax suicide notes.<sup>4</sup> The result of the analysis is an assessment of how likely the questioned document is to be a real suicide note. SNARE distinguishes real suicide notes from control documents with an accuracy of 80%; this accuracy increases to 86% if the real suicide note data is limited to notes of 45 words or less.<sup>4</sup> Mental health professionals, on the other hand, are claimed to identify suicide notes as real or not with an accuracy ranging from 63% to 71%.<sup>5,6</sup>

To date, SNARE has only been used on suicide notes and student writing for the assessment of suicidal ideation.<sup>4,7,8</sup> However, there are other text types that the tool might be useful in analyzing as well, such as the last words of inmates on death row, which are also expressed (either spoken or written) prior to imminent death. Studying last words is a relatively new research field, in part because data is less accessible. This study uses the final statements of death row inmates in Texas from 1982 to 2020, publicly available on the Texas Department of Criminal Justice website.<sup>9</sup>

Applying SNARE to these last words provides an objective quantitative comparison of last words directly to real suicide notes as well as to other potentially overlapping text genres from the control document dataset to obtain a better understanding of the pertinent linguistic features and evaluate the mental state of the inmates prior to imminent death.

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### Quantitative Forensic Linguistics, Last Words, Suicide Note

## D30 Computational Classification of Written Statements as True or False: Comparing Experimental and High-Stakes Data in the Forensic Setting

Angela Almela, PhD\*, Universidad de Murcia, Murcia 30071, SPAIN

**Learning Overview:** After attending this presentation, attendees will better understand the motivation and challenges of the computational classification of written statements as true or false in the forensic setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing empirical evidence on the classification of true and false texts of different nature by means of computational tools.

In the past decade, fields such as psychology and natural language processing have devoted considerable attention to the automatization of the process of deception detection, developing and employing a wide array of automated and computer-assisted methods for this purpose.<sup>1,2</sup> Similarly, another emerging research area is focusing on computer-assisted deception detection using linguistics, with promising results.<sup>3-5</sup> Thus, some computational approaches supervised by experts in the field are considered an efficient way to supplement and support criminal investigators, being of special interest to linguists, jurists, criminologists, and professionals in the field of communications.

Accordingly, in this presentation, attendees will first be provided with a framework introducing the theory that explains the origin of deception, offering an operational definition of the concept and describing key notions like theory of mind, emotional arousal, and empathy. Then, an overall review of the state of the art regarding linguistic cues to deception will be provided, as well as an overview on several approaches to the study of deception and on previous research into its linguistic detection, describing the main controversies in the area.<sup>6</sup> Furthermore, this presentation will draw a distinction between software packages specifically developed for linguistic deception detection, like Witness Statement Evaluation Research (WISER) and VeriPol, and other verbal assessment tools that are widely used for this and many other purposes, such as Linguistic Inquiry and Word Count (LIWC).<sup>7-9</sup>

An empirical study is presented, whose aim is to explore the linguistic cues to deception in written language using WISER, a project using automated text analysis and statistical classifiers to determine the best protocol for computational classification of true and false statements in the forensic-investigative setting. The tool has been tested on ground truth data, which involves the use of data where the researcher knows what the correct answers are, as only with ground truth data can the researcher accurately report its error rate. Specifically, in previous work, the results showed a remarkable difference between the experimental data, in which students were asked to write true and false narratives, and high-stakes data, actual statements from real criminal investigations with non-linguistic evidence of their veracity or falsehood. This result demonstrates that there is a real difference between lies told in an experimental setting (or “laboratory produced”) and lies told in a police investigation.

In the current study, a different set of experimental data was tested against the high-stakes data to determine if the previously reported distinction between laboratory-produced lies and high-stakes lies is replicated. The dataset has already been analyzed with different sets of variables, through the application of Machine Learning and statistical classifiers, which provides a solid basis for comparison.<sup>1,3</sup> Specifically, data were produced by 100 participants, all of them native speakers of English participating through Amazon<sup>®</sup> Mechanical Turk. Subjects were asked to write on three different topics, first voicing their real opinion on the matters in four or five sentences; then, they had to express the opposite of their opinion, explicitly lying about their true beliefs. The corpora do not contain spontaneously produced language, but the Hawthorne effect was minimized by not explicitly telling the participants the ultimate aim of the study. Moreover, a reward provided medium motivation for the participants, as they were assigned with the tasks as an exercise for extra credit.

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### Automatic Text Classification, Deception Detection, Forensic Computational Linguistics

## D31 Deception Data, Mindset, and Validation Testing

Carole E. Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE 19947

**Learning Overview:** After attending this presentation, attendees will better understand how to evaluate methods developed for classifying written statements as true or false. Attendees will be able to assess the deception data in relation to one individual's mindset and how different mindsets affect validation test results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing cautionary evidence regarding the validity and usefulness of different methods for linguistic deception detection.

The ability to discern that statements are false is a much-coveted and highly regarded tool in any investigator's toolkit. This ability, however, is mitigated by the "truth-bias" that humans have: humans want to be told the truth and therefore can easily be fooled by other humans who do not tell the truth.<sup>1</sup>

One approach to overcoming the "truth-bias" is to assert that any subject in an investigative setting is lying and to train investigators to convince the investigative subject that he is lying. This approach is at the core of the Reid Technique, which provides seven different ways for convincing the investigative subject that he is lying.<sup>2</sup> The well-documented problem with this first approach is that it can lead to false confessions, if the investigative subject cannot resist the investigator's methods.<sup>3</sup>

A second approach to overcoming the "truth bias" is to employ "wizards" who, unlike most humans, appear to be able to discern deception better than others.<sup>4</sup> However, wizards are rare, and although popularized by television shows, validation of the "wizards" is weak statistically.<sup>5</sup> In fact, even experienced police interrogators are not perfect deception detectors.<sup>6</sup>

A third approach to overcoming the "truth bias" is to employ a checklist in which an investigator looks for specific patterns that are identified as untruthful. This approach is known as "statement analysis" or "investigative discourse analysis" or Scientific Content Analysis (SCAN).<sup>7-9</sup> While the checklist approach can be systematized, validation testing of its reliability shows that it is weak because the investigator may or may not follow the checklist. In one test of SCAN, for example, four investigators agreed with each other, but they each used a different set of features from the same checklist.<sup>10</sup>

A fourth approach to overcoming the "truth bias" is to employ computer software that classifies a written statement as true or false based on the quantified linguistic patterns in the text that match a statistical model of true or false statements. Since computer software and statistical analysis are not human, they do not suffer from the "truth bias," nor do they suffer from an anti-truth bias. Such algorithms merely, without any skin in the game, calculate the probability that the linguistic features of a written statement are more like the statistical model of truthful or false statements. VeriPol and Witness Statement Evaluation Research (WISER) are both computer programs that have attained similarity reliability in validation testing.<sup>11,12</sup> VeriPol attained 91% accuracy at detecting deceptive statements from a very large dataset collected by the Spanish National Police Corps in Madrid; WISER attained 93% accuracy at detecting deceptive statements from a very small dataset collected by a Midwestern American police department. Both of these results for algorithm-based analysis are based on "high-stakes" data, statements written during police custody.

Empirical results are presented which answer the question: does the mindset of the investigative subject affect the algorithmic classification of true and false written statements? If the investigative subject truly believes that he is telling the truth, can falsehood still be detected? If the investigative subject truly believes that he is telling a lie, can truth still be detected? WISER was deployed on four different datasets that reflect different mindsets: (1) true or false statements to the police, a high-stakes setting; (2) written statements regarding the investigative subject's abduction by aliens; (3) true or false statements written by experienced forensic interrogators; and (4) true or false statements written by students, a low-stakes laboratory setting.

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### Deception Detection, Linguistics, Natural Language Engineering

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## D32 Detecting Linguistic Markers of Religious Extremism in Religious Mosque Sermons: A Case Study of Pakistan

Mariam Dar, PhD\*, *The Institute for Linguistic Evidence (ILE), London, ON N6M 0B5, CANADA*

**Learning Overview:** After attending this presentation, attendees will better understand the role of religious mosque sermons in the radicalization of youth in Pakistan and forensic computational linguistic analysis for detecting linguistic markers of religious extremism in spoken language.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing multilingual English-Arabic-Urdu analysis of spoken language for detecting linguistic markers of extremism for proactive investigations.

In Muslim countries, there are religious sermons every Friday in the local mosques. The young people that go to mosques are vulnerable to being easily manipulated. It is unfortunate that many local religious clerics can promote extremist views from the pulpit, either on purpose or because they have inadequate knowledge of the religion. Religious extremism has been on the rise as shown by the significant increase in stereotypes related to Islam. Religious extremism has been a huge problem, especially in the context of South Asia. Pakistan is among the top five countries affected by terrorism/violent extremism.<sup>1</sup> A fair share of the growing challenge of youth radicalization in Pakistan is attributed to religious mosque sermons.<sup>2,3</sup> The advantages of using these religious gatherings include communication channels that are informal, large groups, and they are cheap and decentralized.<sup>2,3</sup> Extremist religious communication facilitates brainwashing, radicalization, and recruitment to embrace jihad in other countries.

Despite the initiatives by the government to ban and regulate social media platforms as well as mosque sermons, under the Citizens Protection (Against Online Harm) Rules, 2020, a set of regulations on social media content, the radicalization of youth has been a constant threat in Pakistan. If linguistic markers of religious extremism in real-time religious meetings are detected early, they can be very helpful in preventing an act of terrorism and identifying culprits. This approach calls for the use of specialized software that can play an important part in identifying radicalization communication by detecting warning signals/threats associated with such communication. Machine learning techniques can be used effectively to detect “weak signals” and “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” (indicates a desire to identify oneself with previous attackers or a terrorist organization).<sup>4-8</sup> Once these warning signals are identified with the help of linguistic markers associated with them, various tools and techniques can be used for speaker identification, speaker profiling, and the prevention of future crimes.<sup>9</sup> Further, mosques where sermons are not radicalizing can also be detected through the recurrent absence of these linguistic radicalization markers, thereby protecting religious freedom.

In order to study the language, Friday mosque sermons were recorded from the local mosques of three different cities for a period of three months for a total of 36 sermons. The speech data was translated from Urdu to English by a bilingual Urdu-English linguist and analyzed using Automated Linguistic & Assessment System (ALIAS).<sup>10</sup> ALIAS was used to calculate quantitative rates for words related to extremism. This helps to determine which key words or phrases show up repeatedly, which is helpful in objectively defining the basic terminology of extremism. Syntactical analysis is also conducted to determine if there is a correlation between the content (extremist thoughts/ideas/messages) and syntax (what is the syntax in extremist vs. non-extremist phrases/sentences). This presentation showcases the results of quantitatively analyzing the language of Friday mosque sermons to identify trending words related to both moderate and extreme versions of Islam and to apply syntactic analysis for differentiating moderate and extreme sermons.

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### Extremism in Mosque Sermons, Linguistic Markers, Religious Extremism in Pakistan

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## D33 Elementary Analytical Techniques to Enable Verification of Claims and Data That Lie Outside Personal Specialism Comfort Zones

*John Nixon, MBA\*, ARC, Bippus, IN 46713*

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**Learning Overview:** After attending this presentation, attendees will be aware of the common problem of unchallenged acceptance of claims and data. Attendees will learn how to verify or discredit claims and data in almost any specialism via the application of elementary analytical techniques, such as supposition, estimating, bracketing, and basic mathematical analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of an often-overlooked and under-appreciated problem that has a major impact upon daily life, including life within the justice system. This presentation will demonstrate that the timely application of easily applied basic analytical techniques is a time- and cost-effective way to screen out bogus assertions that can often result in wasted resources and even hysteria.

**Summary, Hypothesis, and Proposition:** On a daily basis, we all deal with a multitude of people in a range of roles—politicians, car salespeople, lawyers, etc. Then there are the advertisements and infomercials. Are they lying to you, are they being politically correct, are they giving you only part of the truth (what is omitted is often more important than what is communicated)? Can you believe your doctor when he recommends a new medication, or is his judgment being influenced by an expenses-paid trip to the country club—how would you know, how can you find out? Only a few decades ago, tobacco company executives testified under oath that nicotine was not addictive; it is now widely recognized to be addictive—were they lying or have humans undergone biological change?

Some lies are more enduring than others. Published data from “reliable sources” can be deceptive and is less likely to be challenged; there are lies, damned lies, statistics, and government statistics.

The 2012 Alvarez ruling from the United States Supreme Court focused on interpretation of the First Amendment to the United States Constitution.<sup>1</sup> In short, that ruling essentially interpreted the First Amendment as providing the right to lie. Unfortunately, it seems that many people view it as an obligation, rather than just a right, and it often feels as though we are drowning in lies and deception every minute of every day. So, how do you plough through it all and get to the truth?

Journalists and television commentators frequently proclaim that they have “fact checked” claims made by a corporation or an individual; but in a world of many “alternative truths” just what “facts” can you have confidence in? Did you just watch fake news, real news, or was it even news at all? If you try a little fact checking on the internet for any subject about which you possess little personal knowledge, you will likely encounter dozens of “opinions” and come away more confused than when you started.

Rather than seek out the opinions of others, it is often best to form an opinion of your own. Elementary analysis of the “facts” thrown at you can often reveal more than talking with several experts. Individuals have a duty to exercise due diligence in their personal and professional lives, and becoming proficient in a few basic analytical techniques can help to distinguish valid claims and data from the invalid, thereby avoiding serious problems.

This presentation will present real-world examples of lies and deception and analytical techniques that the average person can employ in order to assess the likelihood that they are being deceived.

### Reference(s):

<sup>1</sup> *United States v. Alvarez* (567 U.S. (2012)).

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### Lies, Deception, Omission

## E1 A Method for the Determination of Canine Olfactory Limits of Detection (LoD) Using a Quantitative Vapor Delivery System

*Lauryl DeGreeff, PhD\**, United States Naval Research Laboratory, Washington, DC 20002; *Christopher J. Katilie, Nova Research, Inc, Alexandria, VA 22308*; *Ryan F. Johnson, PhD, United States Naval Research Laboratory, Washington, DC 20375*

**Learning Overview:** After attending this presentation, attendees will better understand the features and validation of the Trace Vapor Generator (TV-Gen), previously designed, fabricated, and validated by a multidisciplinary team of scientists at the Naval Research Laboratory, for the generation and delivery of reproducible and adjustable quantities of odor for canine testing.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a means to compare canine olfactory sensitivity to instrumental sensitivity.

The canine detector is one tool in an arsenal of commercially available chemical sensing instrumentation or sensors with diverse applications utilized by military, homeland security, and law enforcement. Canine sensitivity, meaning the ability to detect some minimum concentration of odor, is frequently reported as being lower than field detection instruments; however, there is minimal analytical information to support this claim. This presentation will discuss the development of a quantitative method of vapor delivery that will support research aimed at analytically assessing the LoD for canines.

The first attempt at canine LoD measurement was in 1953 by W. Neuhaus, where the threshold of canines for butyric acid was investigated. This study was designed to compare the olfaction systems of canines to humans. A controlled method of odor delivery was used, and canines were found to have a threshold of approximately eight logarithm (log) units lower than humans. However, further comparable studies showed great discrepancies when measuring canine LoD.<sup>2</sup> Although these and more recent studies were generally well designed and provided some necessary insight into canine LoD measurement, some issues with these testing methods were still apparent, yielding inconsistencies in reported measurements and large variations in threshold estimates. Odor delivery and sample introduction methods were thought to contribute to the discrepancies in LoD studies. For instance, the mentioned studies did not account for dilution of the odorant or loss of the odorant due to adsorption to transport materials. These are necessary parameters to consider because frequently when an odor is contained or passes through a material, such as tubing, a portion of the odor will adsorb onto the transport material and result in a lower vapor concentration. Additionally, once odor leaves the source, the vapor plume is diluted by the immediate air, lowering the concentration. Furthermore, it is difficult to control environmental variables. For example, surrounding air can carry the already dilute odor further away from the source, which would result in yet an even lower vapor concentration.

The objective of this presentation is to present an accurate, reproducible, and quantitative method for vapor delivery that will be utilized to measure canine LoD, as well as directly compare canine sensitivity to instrument sensitivity. Attendees will learn about the features and validation of the TV-Gen.

The TV-Gen was tailored to canine testing needs by including a custom canine sampling port and configuring appropriate sample vapor flow and temperatures. Computational fluid dynamics modeling was used to ensure even distribution of analyte vapor in the canine sample port. The generation and quantitation of three probative analytes—isoamyl acetate, 2,4-dinitrotoluene (a volatile component associated with Trinitrotoluene [TNT]), and methylbenzoate (volatile component associated with cocaine)—will be discussed, and the ability to easily adjust the vapor concentration of these analytes will be shown. Known and accurate concentrations of analyte vapors were generated, and the concentrations were decreased linearly by dilution of the aqueous solution or airflow stream. A carryover study also showed minimal-to-no-carryover of analyte in the sample port when switching from analyte to clean vapor streams. Follow-on work using these analytes at increasingly lower concentration will assess the response of canine detectors to determine their LoD.

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### Canine Detection, Limit of Detection, Vapor Delivery

## E2 Documenting Outdoor Skeletal Scatters Using Close-Range Photogrammetry (CRP): Testing the Number of Individual Coded Targets to Improve 3D Model Accuracy

*Morgan J. Ferrell, MA\*, Orlando, FL 32803; John J. Schultz, PhD, University of Central Florida, Orlando, FL 32816*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of methods for improving 3D documentation of crime scenes in wooded environments when using CRP. In particular, this presentation will focus on whether incorporating individual coded targets throughout the scene in addition to photogrammetric scale bars with integrated coded targets improves the accuracy of the final 3D models.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating methods for increasing the overall accuracy of CRP-generated 3D models when recording skeletal scatters in wooded environments.

CRP is commonly employed by archaeologists to record excavations and artifacts, and guidelines are well developed for archaeological applications. However, archaeological sites recorded using CRP are often different environments from those in outdoor wooded crime scenes. Therefore, the CRP methods employed by archaeologists to generate 3D models need to be tested in simulated forensic settings to better understand how photogrammetric techniques can be adapted for forensic contexts. One method for improving model accuracy that needs to be tested in a forensic setting is the application of individual coded targets. There does not appear to be consistency in archaeology regarding whether incorporating individual coded targets is necessary to generate accurate 3D models. Furthermore, archaeologists are often recording excavations with a cleaned, level, and homogeneously textured bottom surface and typically are not working in a wooded environment with a complex bottom surface. As a result, it is unknown if the addition of extra coded targets will improve accuracy of common forensic scenes with complex ground surfaces that are typical of most wooded environments. Therefore, the purpose of this research was to test the applicability of using extra coded targets in conjunction with scale bars that include integrated coded targets in a wooded simulated scenario.

One simulated forensic scene consisting of a slightly scattered composite human skeleton and clothing was constructed in an oak hammock environment with a ground surface consisting mainly of leaves. Cultural Heritage Imaging-calibrated photogrammetric scale bars were placed around the scene, and photographs were taken freehand using a Nikon® D7200 camera from five view angles while moving around the scene. Additional close-ups were also captured of individual bones and joint surfaces. The scene was photographed a total of four times while varying the number of extra coded targets (12, 8, 4, and 0) placed throughout the scatter. Images were preprocessed using Adobe® Bridge, and Agisoft® MetaShape Professional was then used to generate 3D models and orthomosaic maps of the scene. Accuracy of the 3D models was assessed using Root Mean Square (RMS) reprojection error, scale bar errors, and through visual examination of the 3D models.

Overall, all four models were highly accurate, regardless of the number of individual coded targets used. The RMS reprojection error values for each model did not vary significantly, with all four models achieving an error value close to 0.3 pixels. Additionally, the RMS reprojection error did not exhibit a trend that correlated to the number of extra coded targets. For example, Model 4 (zero extra targets) exhibited the lowest error at 0.335 pixels, while Model 2 (eight extra targets) had the highest at 0.371 pixels. However, there was a consistent trend of lowest total scale bar error for Model 1 (0.053mm) with 12 extra targets to the highest total scale bar error for Model 4 (0.142mm). While all four models achieved scale bar errors of less than 1mm, the level of visual error varied between the four 3D models. Additionally, the visual error does not appear to correspond with the number of extra coded targets because the cranium exhibited the least amount of visual distortion in Models 2 and 4, while it exhibited the most severe distortion in Model 1.

This research has demonstrated that using additional coded targets is not required for documenting forensic scenes with complex bottom surfaces when used in conjunction with scale bars with integrated coded targets. A challenge encountered during the documentation of the skeletal scatters was the varying lighting conditions throughout the day, which resulted in shadows. While this issue would be typical of a wooded scene, major lighting issues such as extreme shadows and highlights can be adjusted when preprocessing the images.

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**Close-Range Photogrammetry, Scene Documentation, Forensic Archaeology**

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### E3 Classification of Tires Using Elemental Fingerprinting

*John Lucchi, BS\*, University of Central Florida, Orlando, FL 32826; Dan Gluck, National Center for Forensic Science, Orlando, FL 32826; Larry Tang, University of Central Florida, Orlando, FL 32766; Matthieu Baudelet, PhD, National Center for Forensic Science, Orlando, FL 32826*

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**Learning Overview:** After attending this presentation, attendees will have gained insight into the use of elemental analysis to yield forensic classification of tire trace evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the foundation for a neglected form of evidence: the rubber material left as vehicle tire marks.

Tire evidence is a form of trace evidence that is often overlooked in today's forensics. Tire evidence can often be found at crime or accident scenes, most often in the form of skid marks. Traffic accidents represent a large portion of incidents in the world, covering property damage, injuries, and/or fatalities. In the United States between 2004 and 2018, 5% of traffic cases were fatal hit and runs, where knowledge about the car tire could sometimes be the only information available. While the pattern of the tire skid mark has been used before to link a tire or car to a scene, the widespread use of anti-lock braking systems makes this an almost impossible and often abandoned route of analysis. With this in mind, using the chemical profile of a tire has the potential to link a car or tire back to a scene in which its trace material is found.

Most current research into this topic involves looking at the molecular signature of the tire through pyrolysis-gas chromatography mass spectroscopy. However, there is concern that the conditions of skid mark trace evidence formation will obscure the molecular signal of the tire and will be hard to replicate exactly through analytical methods, possibly making classification impossible. A route to avoid this issue is to instead look at the elemental profile of the tires, which is less likely to be different between tire and skid mark. Looking at the elemental profile is an accepted technique in the current forensics field, which can be obtained in many ways from Energy-Dispersive X-Ray (EDX) spectroscopy to Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) to Laser-Induced Breakdown Spectroscopy (LIBS).

Thirty-two tire samples (18 brands) provided by the Florida Department of Law Enforcement were analyzed using LIBS under argon atmosphere. These samples were taken from the surfaces of tire treads and analyzed using an Ultraviolet (UV) LIBS unit (J200, Applied Spectra). Data were analyzed through Principal Component Analysis (PCA) followed by Linear Discriminate Analysis (LDA). PCA is used to reduce the dimensionality of the data, while LDA serves as the classification method, leading to an accuracy close to 99%. These first results are promising and open the path to the use of tires and their residues as a forensic evidence that has so far been neglected.

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#### Tires, Trace Evidence, Elemental Analysis



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## E4 Sparse Modeling for the Classification of Evidence From Spectral Data

Dayla C. Rich, BS\*, University of South Carolina, Columbia, SC 29208

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**Learning Overview:** After attending this presentation, attendees will have a deeper appreciation and understanding of the role multivariate statistical techniques, in particular sparse methods, can play in the interpretation of spectral data of trace evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how statistical analyses, specifically sparse chemometrics, can aid in classifying evidence from their respective spectral data.

Ultraviolet/Visible (UV/Vis) data of dyed fibers and Fourier Transform Infrared (FTIR) spectroscopy data of lipstick samples will be explored in this presentation. Trace evidence can play a critical role in successfully solving a criminal case by allowing for associations to be made between suspects, victims, and the crime scene. A common technique utilized to analyze the dyes on fibers is UV/Vis Microspectrophotometry (MSP).<sup>1</sup> Further, lipsticks are analyzed through a plethora of techniques. FTIR is commonly used in forensic science and offers a quick and non-destructive method that yields vital information about the chemical nature of the sample.<sup>2</sup> However, classification of these two sample types via spectral analysis (spectral overlay) can be difficult due to the complexity of the data (i.e., large number of wavelengths or wavenumbers in spectral data).<sup>3</sup> Multivariate statistical methods can help in drawing conclusions about qualitative forensic analyses. Sparse chemometrics simplifies the predictive model by shrinking unimportant variable coefficients to zero. Thus, only the most significant variables are retained in the final model.<sup>4</sup> This research achieves sparsity using logistic regression with Least Absolute Shrinkage and Selection Operator (LASSO).

When compared to the more common statistical approach of principal component Analysis (PCA) combined with Linear Discriminant Analysis (LDA), the sparse method outperformed in prediction accuracy. Logistic regression with LASSO achieved 96.6% prediction accuracy for the fiber data set, while PCA with LDA yielded only 89.7% prediction accuracy. For the lipstick samples, prediction accuracy also increased. PCA with LDA achieved 60.0 % accuracy, while logistic regression with LASSO achieved 66.7% prediction accuracy. Limiting predictive models to only consider the most critical variables (sparsity) also greatly enhances model interpretability. Rather than considering hundreds of wavelengths or wavenumbers, only the most important and informative variables are retained in the model. As statistical analyses increase in popularity and become more prominent tools in the forensic science community, sparsity can help overcome the limitations of traditional modeling techniques and aid in the classification of various types of trace evidence.

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### Sparse Chemometrics, Fibers, Lipsticks

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**E5 Differentiating the Thickness of Black Plastic Bags Used in Bomb or Weapon Wrappings:  
A New Palm-Sized Digital Micrometer Approach**

*John Z. Wang, PhD\*, California State University-Long Beach, Artesia, CA 90701*

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**Learning Overview:** After attending this presentation, attendees will have learned how to differentiate black plastic bags by thickness using a new palm-sized digital micrometer. Black plastic bags are often used to wrap a bomb (e.g., the Madrid Bombing Incident) or a weapon (e.g., in many murder cases). While the Gas Chromatography/Mass Spectrometry (GC/MS) has been used to differentiate the elemental components of plastic bags, the method requires complicated techniques, longer times for sample preparation, advanced examination skills, and is destructive testing. Therefore, for a more practical application in the field for a preliminary source identification, a quick, in-field, and non-destructive device is much needed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a cutting-edge method of measuring the thickness of plastic bags in a quick, in-field, and non-destructive manner. Attendees will learn how the method may assist in investigating bomb- or weapon-related cases as a supplementary ability and competence.

Due to the chemical complexity of plastics, it is still a challenge to compare an unknown sample from a scene to a known sample for a source identification. Therefore, for a more practical application in the field, a quick, in-field, and nondestructive device is much needed for preliminary source identification. A physical approach may provide a new solution to the examination of trace evidence. A new type of palm-sized digital micrometer (different from a traditional micrometer screw gauge) is able to measure the thickness of collected samples ( $n=10$ ) at a micro-level ( $\mu\text{m}=1\times 10^{-6}\text{m}$ ) and can reach up to one place after the decimal point of the  $\mu\text{m}$  unit.

This study employs a semi-experimental design: (1) ten black plastic bags were purchased as samples ( $n=10$ ) from various stores; (2) each sample was cut into a 3mm square (similar to tiny pieces of black plastic from a bomb scene); (3) the micrometer was used to measure the thickness on three different spots on each sample; (4) the three results (per sample) were recorded and averaged to represent the thickness of that particular plastic sample; (5) the same process was repeated for another set of ten samples; (6) the final means of each thickness was compared between two sets; (7) correlations (the final means of each plastic sample) between two sets were identified; and (8) if the means between two sets were quite close to each other, it may suggest that the micrometer can be used to differentiate different types of black plastic bags as a quick, in-field, and non-destructive method.

This presentation will demonstrate the whole process of measuring the two types of black plastic bag samples for the attendees to watch via the Zoom connection with the following focuses: (1) the new palm-size digital micrometer for potential field and lab work (without mentioning the brand and the manufacturing); (2) the black plastic sample (3mm by 3mm); (3) the measuring action (about 50 seconds per piece); and (4) the two sets of the means from the two samples.

In conclusion, this novel method using a palm-sized digital micrometer may suggest a new tool to: (1) differentiate various types of black plastics by one of the physical properties of black plastic bags, namely the thickness; (2) help identify a possible source of a known sample (if available); and (3) make a final decision among exclusion, inclusion, identification, or inconclusive to promote accuracy, precision, and specificity in the forensic sciences.

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**Micro-Measurement, Plastics, Thickness**



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## E6 An Initial Investigation Into the Effects of Lacquered Ammunition on Toolmark Transfer

*Nicole Marie Groshon, MS\*, Jersey Village, TX 77065*

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**Learning Overview:** After attending this presentation, attendees will be aware that lacquer is commonly used as a waterproofing agent and rust protection agent for ammunition. When a firearm is shot, the lacquer on the ammunition may flake and bubble, thus interfering and even hiding the toolmarks used for comparison. A universal protocol for examining lacquered ammunition should be considered when handling this type of evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that the lacquer used to seal cartridges may interfere in the toolmark transfer process.

When a firearm is discharged, the bullet and cartridge case acquire unique microscopic markings called toolmarks. Firearm forensics involves comparing two objects with similar toolmarks. These comparisons typically involve the bullets or cartridge cases found at a crime scene to ammunition that was test-fired with the suspected weapon. Historically, the primer faces of fired cartridge cases have been examined using a comparison light microscope and entered into a database. In recent years, agencies and laboratories have been transitioning to 2D and 3D imaging to aid in faster comparisons with the help of automated search software programs. The database assigns a match score for the cases, and the examiner makes a final comparison of the evidence and test-fired ammunition to determine if there is a true identification.

One troublesome feature firearms examiners may encounter is the lacquer that is commonly used to seal the primer of cartridges. The purpose of the sealant is to prevent moisture from making contact with the gunpowder inside the cartridge, which would render it useless. So far, little research has been done to test the effects of lacquer on the transfer toolmark process on fired ammunition. Whether an examiner prefers using the comparison light microscope or virtual imaging, the lacquer may need to be removed at some point during the examination as it tends to chip and flake off in patches, thus completely changing the topography of the surfaces compared. There has been concern, however, that the cleaning process may destroy some of the individual characteristics that are also necessary for comparison. In this study, researchers examine several different firearms to determine if lacquer affects the toolmark transfer process.

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### Lacquered Cartridges, Toolmarks, Cadre



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## E7 Mitigating Entomological Hazards in Scene Investigation

*Michelle R. Sanford, PhD\*, Harris County Institute of Forensic Sciences, Houston, TX 77054*

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**Learning Overview:** After attending this presentation, attendees will have increased awareness of insects and arachnid pests that present potential scene hazards and how to avoid them, as well as basic principles of integrated pest management.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing investigator ability to reduce the annoyance and disease transmission potential of insect and arachnid pests that may be encountered during scene and case work.

Scene investigation presents many potential hazards. One hazard category, which may not always be at the forefront until well into the investigation, are the insects and arachnids that have the potential to bite, annoy, or transmit disease to investigators. Scene investigations often involve human habitations that are prime locations for insect and arachnid pests ranging from annoyance, to mechanical vectors, to established pathogen/parasite vectors. An appreciation of the risks associated with insect and arachnid pests at the scene is the first step to avoiding interaction with them and any potential pathogens they may be carrying. At one end of the spectrum are pests that represent mostly an annoyance hazard because their bites or stings that may trigger discomfort (unless an individual is allergic) but do not transmit pathogens. Pests in the annoyance category include fire ants, wasps, bees, pus moth caterpillars, black widow spiders, scorpions, centipedes, and bed bugs. In the intermediate category, pests represent a potential for mechanical transmission of pathogens or parasites and include such pests as cockroaches and non-biting filth flies. The other end of the spectrum includes pests that typically require blood for their life cycle and have the potential to be part of a pathogen/parasite transmission cycle. Some of the common member pests in this group include mosquitoes, ticks, biting flies, chigger mites, lice, and fleas. Identification, avoidance, and repellent practices at the scene and post-scene procedures can assist in reducing the annoyance and disease transmission potential of these pests.

Simple procedures can be implemented to avoid carrying back pests and/or to repel them while at the scene that can help reduce investigator interactions with them. Such methods of the judicious use of mosquito repellents, pant leg taping techniques, clothing treatments, personal protective equipment use, and just changing clothing can all be strategies useful in reducing pest interactions. In this presentation, the use of these techniques will be demonstrated. Additionally, principles of integrated pest management can facilitate the control of these pests if they inadvertently make their home in the office or lab. These principles are well established in pest management for other fields and could be adapted to the forensic investigation office or lab.

In this presentation commonly encountered scene insect and arachnid pests and the potential risks they may pose to investigators will be displayed in order to facilitate identification in the field. Additionally, methods to reduce interaction at the scene and procedures to take upon returning from the field will be discussed.

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### Entomology, Disease Transmission, Avoidance

## E8 A Temporal and Spatial Analysis of Firearm Deaths in Harris County, Texas: 2009–2020

*Stacy A. Drake, PhD\**, Texas A&M University, Houston, TX 77030; *Ned Levine, PhD*, Ned Levine and Associates, Houston, TX 77025; *Nimesh Chandrakant Shah, MPH*, Texas A&M University, College Station, TX 77843; *Alicia Medina, BS*, Harris County Institute of Forensic Sciences, Houston, TX 77054; *Yijiong Yang, BM, MHA*, The University of Texas Health Science Center at Houston, Houston, TX 77054; *Dwayne A. Wolf, MD, PhD*, Harris County Institute of Forensic Science, Houston, TX 77054

**Learning Overview:** After attending this presentation, attendees will be able to understand the temporal and spatial dimensions of firearm suicides and homicides. This analysis identifies internal and external risk factors and areas to target prevention. Attendees will be able to summarize the findings within this large geographic region and translate to their own jurisdiction.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a template for the application of temporal and spatial methods to forensic data to identify areas for prevention and to aid in forensic staffing decisions.

The purpose of the retrospective longitudinal study (January 1, 2009–June 2020) was to address the following gaps: (1) understand how firearm suicides and homicides vary over time within a single large geographic region; (2) identify concentrations of firearm suicide and homicide deaths; (3) examine temporal variations in firearm suicides and homicides by year, season, day of week, and time of day; and (4) examine socio-economic and risk correlates for firearm suicides and homicides by small geographical areas (census block groups).

Analyses included bivariate and multivariate statistical analyses to examine firearm death differences over time. Hot spot analysis and multivariate non-linear spatial regression was conducted of the suicide and homicide firearm deaths.

Of the  $N=6,979$  firearm events involving death, 646 involved more than one firearm death, of which 226 were homicide-suicide and 420 multiple homicides. Overall, 3,087 were classified as suicide (155 in custody), 3,797 as homicide (198 in custody), 31 as accidental, and 64 were undetermined. The age distribution of firearm-related homicide and suicide deaths was 0–14 years (111), 15–24 years (1,668), 25–64 years (4,468), and 65+ years (637). The total number of male and female decedents was 5,920 and 964, respectively. Race/ethnicity included non-Hispanic White (2,517), Black (2,285), Hispanic (1,815), and other (267); the latter includes Asians, American Indians, multi-ethnic, and unknown.

Correlates of suicidal firearm deaths included non-Hispanic White ethnicity, prior psychiatric history, and veteran status. Correlates of homicide firearm deaths included Black and Hispanic ethnicity and prior history of gunshot wound injury. Circumstances involving multiple firearm deaths, interpersonal violence, and at-work deaths had a higher incidence of homicide than suicide.

The overall trend is a steady increase in both homicide and suicide firearm deaths. June of 2014 had a decline in firearm deaths and an increase in firearm homicide deaths after June of 2019. Firearm-related homicides were higher at nighttime, while suicide firearm deaths were highest in the daytime. Homicide deaths were highest on Friday night, Saturday night, and Sunday early morning, while suicides were highest on Monday. Firearm homicides were generally located closer to downtown Houston, TX, whereas firearm suicides were generally located farther from downtown. There was some overlap in several areas for which future research and prevention efforts can be targeted. The results have implications for practice and prevention.

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### Forensic Science, Suicide Firearm Deaths, Homicide Firearm Death

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## E9 Getting Unstuck on Tape Testing

Leslie Parke, BS\*, Signature Science, LLC, Austin, TX 78759; Kathleen Q. Schulte, MS, Signature Science, LLC, Austin, TX 78759; Curt Hewitt, Signature Science, LLC, Austin, TX 78759

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**Learning Overview:** Handling tape during the DNA extraction process has long been a challenge, but the value of this type of evidence makes it worth the headaches associated with handling and processing tape. Most labs are either “stuck” with taking cuttings of this hard-to-handle matrix, wrangling it into an extraction tube without it getting stuck, or swabbing it and potentially leaving behind DNA from touch depositions on the adhesive side. The goal of this presentation is to discuss a new method that solves these problems and improves overall DNA results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that using this new method for processing tape can increase the forensic value of the adhesive sides of tape samples and improve DNA recovery over current methods.

The analysis of adhesive samples presents a significant logistical challenge to forensic laboratories. First, manipulating tape carries a significant risk of contamination as material from the environment or the casework analyst comes into contact with the adhesive. In addition, handling challenges also create the possibility of stretching or creasing the tap, which risks altering fingerprint analysis. Finally, current Latent Print (LP) and DNA methods do not allow for testing of both signatures on the same sample. Dyes or compounds used to visualize latent fingerprints can act as inhibitors for DNA analysis, or their application processes can wash or brush away a portion of the DNA. Conversely, DNA extraction is a destructive process for any LP patterns on the surface. Ultimately, the factors associated with tape handling, LP visualization, and DNA analysis often work against each other, forcing forensic laboratories to prioritize/triage investigating specific signatures and analyses over others.

To solve this problem, Signature Science, LLC (SigSci) developed a method, termed Tape Analysis For Forensic Identification (TAFFI™), to allow the processing of adhesive surfaces for both LP and DNA on these substrates. The TAFFI™ workflow starts by attaching the non-adhesive side of evidentiary tape to a backing strip that holds the tape flat and keeps the adhesive side accessible for print imaging and DNA collection/extraction. The backing strip can also be applied after developing the fingerprints in order to accommodate samples where the prints of interest are located on the non-adhesive side of the tape.

Fingerprint visualization on the tape is accomplished via cyanoacrylate fuming followed by nebulization of a fluorescent dye mixture selected to reduce potential downstream inhibition during DNA analysis. Following LP development, the backing strip/tape sample is rolled up, then inserted into a spin basket. The stippling on the backing strip creates a slight gap between the rolled layers of the adhesive side of the tape. This gap allows for improved access of the collection/lysis buffer to the adhesive side of the tape while minimizing the volume of buffer needed to contact the tape. As a result, the DNA extraction efficiency is maximized and sample dilution is minimized.

Tests that were conducted using three different donors who deposited three replicates on four different types of tape produced usable Short Tandem Repeat (STR) profiles in 92%, 83%, and 50% of the samples (calculated by donor due to the variability in DNA deposition between the individual donors). When using a known amount of genomic control material (i.e., positive control applied to tape samples), DNA recoveries ranged from 15% to 34% across the four tape types and were twice as high as the gold standard method. For LP quality, Sears scores of 3 or 4 were observed for almost 90% of the donor samples, and 20+ minutia were observed in 72%.

The TAFFI™ approach has been demonstrated to reliably produce successful results when processing the same tape sample for both LP and DNA signatures. With this novel approach, the case triage process no longer requires the laboratory or investigator to pick between LP and DNA signatures when processing tape samples. Overall, the LP component of the TAFFI™ workflow performed comparable to the current gold standard methodologies, and the DNA component consistently showed higher DNA recoveries, higher average DNA yields, higher average peak heights, and more consistent results when compared to the current gold standard method.

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### Tape, Extraction, Adhesive

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## E10 Forensic Podiatry—An Overlooked Science in the Analysis of Feet, Footprints, Gait, and Tracks

*Kewal Krishan, PhD\*, Panjab University, Chandigarh 160 014, INDIA; Michael S. Nirenberg, DPM, Friendly Foot Care, PC, Crown Point, IN 46307*

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**Learning Overview:** After attending this presentation, attendees will have learned the scientific principles of the important and emerging forensic science subdiscipline of forensic podiatry, which pertains to the examination, interpretation, and evaluation of pedal evidence (in particular, footprints, footwear, gait, and tracks) often encountered at crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic scientists insight into and a greater understanding of additional methodologies and tools available to them—either directly or through consultation with a forensic podiatrist—to assist in the analysis of footprints, footwear, tracks, and human remains, and importantly, an understanding of how these principles may be applied to a wide—and growing—number of other scientific and forensic specialties.

Forensic podiatry is a comparatively new scientific subdiscipline of forensic science that emerged in the 1970s through anecdotal observations. Now, 50 years later, its principles are grounded in substantial, researched science. Forensic podiatry has been defined as the application of sound and researched podiatric knowledge and experience in forensic investigations, to show the association of an individual with a scene of crime, or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot.<sup>1</sup>

This presentation will present the utility of an overlooked forensic science and advance to forensic scientists an additional, powerful tool for their armamentarium, which can be directly utilized for analysis of pedal evidence associated with crimes or as an adjunctive application, supplementing a growing number of other scientific and forensic specialties.

Forensic podiatry principles are used to establish identification and biologic profiles in crime scene investigations when foot-related and gait evidence is recovered from the crime scene, such as individual footprints, tracks of footprints, an item of footwear, or when surveillance video captures the perpetrator's gait (i.e., the way they walk). The need to establish the identity of dismembered remains may also arise, not only in the criminal context but in mass disasters or mass fatality incidents. In this regard, forensic podiatry methods can assist in identification from the decedent's foot and its parts. Beyond the criminal context, forensic podiatry principles have utility in other scientific areas, including medicine, biomechanics, engineering, biology, and anthropology.

The scientific methodologies developed within forensic podiatry can be utilized to analyze footprints and tracks—gait patterns—recovered at crime scenes or associated with crimes. Using such forensic podiatry tools will allow the examiner to determine activities that were carried out by persons during the crime and also allow the linking (or unlinking) of individual footprints to suspects.

Forensic podiatry procedures of footprint analysis frequently entail a systematic identification of features, such as corns, pits, ridges, humps, creases, hammertoe deformity, an extra toe, missing toe in the foot impression, and flat footedness; as well as morphologic and anthropomorphic characteristics, such as linear measurement methodologies, to provide estimates of stature, sex, body weight, and other biologic profile attributes. Tracks found at crime scenes can be analyzed through forensic podiatry's gait analysis principles, which include evaluating such measures as step and stride length and base of gait.

### Reference(s):

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### Forensic Podiatry, Crime Scene Investigation, Identification

## E11 Forensic Gait Analysis—Scientific Foundations, Applications, and a Case Study

Michael S. Nirenberg, DPM\*, Friendly Foot Care, PC, Crown Point, IN 46307

**Learning Overview:** After attending this presentation, attendees will understand the scientific foundations, limitations, and current methodology of forensic gait analysis as well as the potential applications of forensic gait analysis to other forensic and scientific specialties and precautions to be aware of when utilizing gait. These areas of consideration will be shown through a detailed review of a case study involving the identification of the criminal of a violent armed robbery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by imparting insight and understanding into the scientific principles and methodology of the new, growing field of forensic gait analysis while explaining the limitations of gait in the forensic context and recognizing the growing number of applications of forensic gait analysis to other scientific and forensic specialties.

Gait—the individual way that people walk or step—has been used by laypersons to identify criminals since 1839. However, the modern, scientific use of gait analysis in the forensic context began in 2000 when a podiatrist used gait analysis to link a suspect to a crime. Since then, striking advances and standards—from the robust United Kingdom’s *Forensic Regulator’s Gait Analysis Code of Practice* to reliable, scientific methodologies—have developed in the new, growing field of forensic gait analysis.<sup>1</sup> This strong, structural framework has enabled forensic gait analysis to become an extremely valuable tool in criminal investigation and prosecution, with applications emerging in medicine, anthropology, digital science, engineering, biology, and other sciences.

Recent research has established a standardized methodology to analyze and recognize features of gait among individuals that offers significant validity, reliability, and accuracy.<sup>2-4</sup> To that end, forensic gait analysis has played an important role in over 100 criminal cases in the United Kingdom and has been increasingly accepted as relevant, admissible evidence in the United States.

Forensic gait analysis allows investigators to assist law enforcement by linking (or unlinking) suspects to crimes by analyzing the way in which a person of interest walks or their *gait* as valuable legal 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—a suspect may be linked to the criminal activity based on his or her gait.

Principles of forensic gait analysis are now being utilized by other forensic and science disciplines. For example, the use of footprints and foot tracks found at crime scenes can be analyzed in conjunction with surveillance video to enhance the assessment of the perpetrator’s gait, either for comparison with a suspect or to create a biologic profile. With video surveillance increasing and phone technology that allows for the recording of crimes becoming more ubiquitous, the need for forensic gait analysis is certain to grow and, with it, the increased application of forensic gait analysis to other disciplines.

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### Forensic Gait Analysis, Gait, Video Comparison



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## E12 Variation in Plantar Pressure Distribution Among Different Body Mass Index (BMI) Categories: Forensic Implications

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**Learning Overview:** After attending this presentation, attendees will comprehend the forensic application of correlating plantar pressure distribution with different BMI categories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the existing knowledge of forensic podiatrists in interpreting the body size of an individual from the plantar pressure distribution of static and dynamic footprints that may be recovered from the crime scene.

Bare footprints, either static or dynamic found as physical evidence at the crime scenes, can provide a connection between the crime and the perpetrator. The masking of the footprint is yet to become prevalent among the criminals; thus, some of them still go unshod while committing a crime. Unlike in India, Western countries where the culture of wearing shoes predominates, the presence of bare footprints is rare although there are instances in the West where there is a possibility of recovering footprints, as is commonly seen in cases of sexual assault or where the perpetrator's mistaken knowledge of footwear identification can lead him/her leaving bare footprint marks at the crime scene. The real value of a bare footprint is when it can be compared with suspects' footprints to establish individualization. Similar to fingerprints, bare footprints represent different levels of uniqueness and individuality depending upon numerous features. Morphologically, the footprint may be normal, flat, curved, or may have any intermediate shape depending on the weight of the body. It may be calculated by analyzing the footprint parameters such as footprint length and breadth, footprint contact area, footprint index, arch index, heel-ball index, etc. In addition, due to the highly associated nature of footprints with stature and body weight, it would not be wrong to state that a correlation between footprints and BMI may also exist.

However, limited forensic studies are available on approximately estimating BMI from static and dynamic plantar pressure distributions. Plantar pressure distribution may be exclusive to each individual and imparts knowledge about the structure as well as function of the foot. In the absence of any set standards, the identification of the criminal becomes difficult. This scientific gap has encouraged the present research work. The main objective of the investigation was to study the plantar pressure distribution with respect to BMI and body size. The study is based upon a random sample of 461 young adults (230 males, 231 females). The target group was identified. The students were instructed about the proper procedure prior to obtaining the footprints from both the feet while standing and walking using standard protocols. The footprints were taken from each participant using an inking method for comparison purposes. Upon examination, all the length measurements (T-1, T-2, T-3, T-4, and T-5) and footprint index showed statistically significant differences among all the three BMI group individuals, but width at ball, width at heel, arch index, heel-ball index, and footprint contact area did not show statistically significant differences. The footprint in terms of arch index indicated the presence of morphological differences between normal weight and obese individuals, with the obese category having a greater tendency toward lower arches or flat feet compared to normal and pre-obese individuals. It was inferred from the results that footprint morphology varied with different BMI categories. Overall, the study concludes that different BMI categories may be estimated by studying the footprint morphology of the plantar area of the foot when the foot makes contact with the ground during standing and walking. As a result, variations in the footprint was observed. However, while conducting this study, certain precautions were followed in the controlled conditions, which will be discussed in the presentation.

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### Forensic Podiatry, Plantar Pressure Distribution, Footprints

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**E13 Footprint Analysis: Data From North India Study Suggests New Features for Individualization and Biologic Profiling**

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**Learning Overview:** After attending this presentation, attendees will have learned the usefulness of the individualistic and unique characteristics of footprints, the foundations and principles of footprint analysis, and how to apply this science to the evaluation of footprints associated with crimes, conduct research in this area, and apply this knowledge to other specialties.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the methods of analysis of footprints in terms of unique and individualistic characteristics that emerged from research on a large North Indian sample. This information will provide forensic scientists with a thorough understanding of new features and characteristics to consider when analyzing footprints in criminal matters, creating biologic profiles, while emphasizing the limitations of such analysis, and avenues to apply this knowledge and its methodologies to other scientific specialties and pursuits.

The modern analysis of footprints in the forensic context began in the 1970s and has made striking and noteworthy advances—progressing from anecdotal reports to reliable, substantial, scientific methodologies. These principles of footprint analysis are extremely valuable in identifying individuals associated with criminal activity. In addition, the principles have applications that may be applied to medicine, anthropology, engineering, biology, and other sciences.

This presentation introduces the findings and practical applications of the research conducted on an adult Gujjar population inhabiting the Sub-Himalayan region of northern India and on the students of a university in northern India as part of a significant investigation funded by the University Grants Commission of New Delhi. This research analyzed adult footprints with the objective of determining new features and characteristics that could be applied in the identification of individuals and in creating biologic profiles.

The research was conducted on a group of 700 adults (500 males and 200 females), ranging in age from 18 to 30 years. This study analyzed important features of the subjects' footprints, including some lesser-considered characteristics that had not been previously examined in a comprehensive manner. These features included biomechanical, morphological, and dermatological aspects, including humps in the toe-line, phalange marks, pits, crease marks, and cracks.

This study also evaluated anthropometric measures, such as relative lengths of the toes, which provide information on their morphology. These findings resulted in the development of a systematic method of categorizing footprints. The research showed that footprints may be classified on the basis of the relative morphological lengths of the first, second, and third toes. These findings suggest that partial footprints can provide valuable information, particularly in forensic matters where a partial footprint at a crime scene may prove adequate to be linked (or unlinked) to a suspect or a person of interest.

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**Forensic Podiatry, Individualistic Characteristics, Biologic Profiling**

## E14 The Proper Identification of Acute Injuries Through Follow-Up Appointments

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**Learning Overview:** The goals of this presentation are to: (1) identify common medical mimics found in the adult sexual assault population; (2) understand how to individualize follow-up recommendations; (3) recognize opportunities for clinical practice change related to re-examination; and (4) describe the role of re-examination in reducing the likelihood of misinterpretation of findings and development of forensic opinion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by empowering the clinical forensic examiner with the knowledge to correctly identify and assess injury, recognize the importance of re-examination of injury, and the role of re-examination in the development of accurate forensic opinions.

Forensic nurse examiners can be apt to experiencing difficulties in accurately identifying and interpreting acute injuries in the adult sexual assault patient. It is known these challenges exist due to mimics caused by natural variants, previous physical trauma, and disease processes.<sup>1,2</sup> It has been noted that misidentification of injury can in fact occur in adult sexual assault medical forensic exams.<sup>3</sup> However, when coupled with medical and assault history, re-examination of non-specific injury is more likely to lead to accurate interpretation of findings.<sup>4</sup>

A literature review on the topic revealed only five useful articles between the years of 2009 and 2020 highlighting the importance of increasing interest in research and programmatic application of this practice among clinical professionals. Mimics can pose significant challenges in interpretation and implication of findings.<sup>5</sup> Improper identification of injury can negatively impact development of accurate forensic opinions.<sup>6</sup> Though mostly found in the pediatric literature, it is well known that misinterpretation of findings can lead to misdiagnosis, missed treatment opportunities, and potentiate inaccurate legal testimony.<sup>7</sup> It is commonplace for other disciplines of medicine and nursing recognize the importance of follow-up care. Emerging best practices suggest that injuries and disease recognized during a sexual assault medical forensic examination are no different.<sup>8</sup> Despite this, usually due to lack of resources, some sexual assault forensic programs do not routinely re-examine injuries.<sup>9</sup> Through re-examination and serial photography, the forensic nurse examiner gains the benefit of observing the healing process and adds depth to their opinion by dispelling or verifying potential alternate causes.<sup>10</sup>

This presentation will focus on three categories of injury including ano-genital, oral, and cutaneous types. Through case examples, the benefit of re-examination in determining acuteness and relatedness of injury to a reported assault will be demonstrated. Aside from re-examination, the contributory components found to be influential in interpretation of findings include past medical history, assault history, time elapsed since assault, location and type of injury, anticipated healing pattern, and consideration of other potential mimics. In conclusion, practice implications include improved approach and management of potential injury and interpretation of injury.

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### Injury, Re-Examination, Accuracy

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## E15 Johannesburg Identification Unit: Identifying the Unidentified Deceased at a South African Medicolegal Mortuary

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**Learning Overview:** The goal of this presentation is to introduce attendees to the work being performed by the Johannesburg Forensic Pathology Service, which is legally mandated to perform postmortem examinations on all cases of unnatural deaths in the greater Johannesburg metropolitan area in South Africa.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting the results of the Johannesburg Identification Unit over the period of January 2018–July 2020. The following data is presented: the total number of unidentified decedents, their demographics, the frequency of successful primary identifier collection, the number of subsequent positive identifications, and the nationality of positively identified decedents.

Unidentified decedents can comprise up to 10.0% of the total number of annual admissions at this medicolegal mortuary. These decedents are given a state-sponsored burial (“pauper burial”) without an identity, which has serious legal, humanitarian, and financial implications. To address these multiple aspects, the Johannesburg Identification Unit was pioneered in 2016 through a collaborative effort between the International Committee of the Red Cross, the Gauteng Southern Cluster Forensic Pathology Services, the University of the Witwatersrand, and the Victim Identification Center of the South African Police Services. The Unit’s role is to perform secondary examinations for identification purposes. This examination involves collecting various secondary identifiers, DNA samples, taking photographic and radiographic images and fingerprints; and using the expertise of various specialists.

Over a period of 31 months, a total of 8,560 medicolegal autopsies were performed at the Johannesburg Forensic Pathology Service. Unidentified decedents comprised 8.1% ( $n=693$ ) of all cases, which is an average of 22.4 unidentified decedents admitted to this medicolegal mortuary per month (excluding skeletonized remains, neonates, and non-viable fetuses). The Johannesburg Identification Unit documented postmortem data from 385 (55.6%) unidentified individuals during this period, of which the demographics comprised mostly of adult (100%), Black (94.5%) males (91.7%). Samples for DNA analysis were successfully collected from most cases in the form of hair (96.4%;  $n=371$ ), blood (92.2%;  $n=355$ ), and nails (90.1%;  $n=347$ ). Through the multidisciplinary approach toward identification and the efforts of the Johannesburg Identification Unit, 87 persons (22.6%) were subsequently positively identified. Fingerprinting was the most successful method of identification (98.9% of cases;  $n=86$ ) that was collected, either completely or partially, from 65.5% of the unidentified cases ( $n=252$ ). A single positive identification was facilitated through DNA analysis. The nationality of over half of the positively identified decedents was confirmed to be South African (52.9%;  $n=46$ ) and, in 36.8%, the nationality was not known. Of the remaining positively identified individuals, their nationalities were confirmed to be from other African countries, namely: Zimbabwe (5.7%), Uganda (1.1%), Mozambique (1.1%), Malawi (1.1%), and Sudan (1.1%). Collecting as much postmortem data to aid in an eventual identification ensures that unidentified decedents are treated with dignity and respect. Through the collaborative efforts of all the agencies involved, the impact of the work of this Unit is vast—not only for South African authorities but, most importantly, for families.

The Johannesburg Identification Unit is also providing training and workshops to other forensic pathology facilities in South Africa, which adopt and adapt the program to their individual facility’s identification challenges and resources. It is hoped that such a program can be expanded to other countries within Africa.

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### Unidentified, Migrants, South Africa

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**E16 Elder Abuse in Northeastern Italy—Health Care Providers’ Perceptions and Medicolegal Perspectives: A Survey of the Phenomenon in Two Italian Hospitals**

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**Learning Overview:** After attending this presentation, attendees will be aware of the results of a survey performed to study the perception of health care workers about elder abuse and the medicolegal consequences of the low perception of the phenomenon in the daily clinical practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by alerting on the importance of reporting abuse to authorities and providing guidelines that can help health care workers: (1) protect the victim, and (2) prevent the abuse from happening again.

Elder abuse is expected to grow in the future with the aging population. The purpose of this study was to analyze health care workers’ perceptions, attitudes, knowledge, and experience about elder abuse and neglect in two hospitals in northeastern Italy, as well as their ability to properly report the abuse and eventually identify the needs of workers in order to improve or change the situation. An anonymous 22-question survey was distributed to all health care workers from two different hospitals in northeast Italy who belonged to departments with a high rate of elderly discharge and to those of emergency rooms. Workers were asked about their perception of the environment, signs and risk factors regarding elder abuse, and their own prior experience with the elderly who suffered of abuse. Descriptive and assessing association analyses were carried out. Questionnaires collected were 209, and the response rate was 46%. Health care workers’ perceptions about elder abuse is overall low: the contexts recognized (familiar, institutional, and social contexts) and the types of elder abuse (physical, psychological, negligence, economical, pharmacological) were very similar to the literature data, but only 6.7% of the health care workers recognized all the possible signs of abuse.

What stands out is not that all the risk factors of abuse in the elderly were recognized and that even though it is known that women are the most common victims of elder abuse, as regards the female sex, this was indicated in only 14% of the questionnaires. On the other hand, there were no differences in perception within the various professional categories (doctors, nurses, socio-health workers). More than half of health care workers had nearly one case of elder abuse in their working activities; some had more than ten cases in their activities, especially workers from emergency rooms. However, many of them did not know how to treat and protect the victim: only 15.7% reported the abuse to authorities and 24.6% activated a social support. However, more than half (65.7%) of the workers usually asked for advice from a supervisor or a colleague. Even if it is a duty based on Italian law to report abuse to the authorities, the answers given demonstrated that health care workers have difficulties to report; therefore, a creation of guidelines is required in order to help them. Available information and education are also poor. The results showed that the knowledge of health care workers about elder abuse is still superficial and limited, only ahead of clear signs and risk factors. Moreover, there is a lack of confidence in reporting abuse to the authorities. There is also a general lack of knowledge about elder abuse and mismanagement by health care workers, but there is an evident interest if updates are proposed.

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**Elder Abuse Perception, Elder Abuse Recognition, Health Care Workers**

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## E17 Murder on the Cruise: The Micki Kanesaki Case and the Scientific Evidence Found

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**Learning Overview:** The goal of this presentation is to provide attendees with knowledge of the famous Micki Kanesaki case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the evidence of the postmortem investigations performed by the Italian forensic team that conducted the autopsy.

The analysis of the case showed that the couple's relationship was marked by continuous quarrels for which, in 2006, the spouses reconciled and decided to take a cruise together to reconstruct their romantic relationship. The American tourist Micki Kanesaki ended up in the sea on the night between May 25 and May 26, 2006, while on a cruise in the Mediterranean, aboard the *Island Escape*. The forensic analysis of the circumstantial data showed that around midnight the couple returned to the cabin drinking, according to the husband, the remaining wine and taking a pill to help them sleep. The husband woke up at 4.30 a.m. Noting the absence of his wife, he prepared to look for her.

On May 27, 2006, the body of a woman corresponding to the description of the passenger who disappeared from the ship was found at 4.00 p.m. The body was transferred to the Port of Vibo Valentia, Calabria, Italy, on May 28, 2006. On this date, the forensic team of the Legal Medicine of the University "Magna Graecia" of Catanzaro intervened and was mandated by the public prosecutor to perform the autopsy. The corpse was dressed in a blue undershirt, a white bra, green trousers such as pajama bottoms, and a blue cotton slip. The body presented initial putrefaction with greenish color of the skin and initial saponification. The corneas appeared opaque and lividity was not imprintable, with no rigidity. At autopsy, widespread injuries were found, including: (1) a collar-shaped ecchymosis with excoriation of II-III degree and interruption of the same at the level of the jugulum; (2) an excoriated ecchymotic area on the anterior surface of the left arm; (3) another bruise with excoriation on the left arm; (4) another excoriation on the thoracic surface and on the anterior surface of the right thigh; and (5) an ecchymotic and excoriated area in the sub-umbilical region.

The internal injuries found at autopsy showed intense hemorrhagic infiltration on the right occipital region. On the right frontal region, there was an area with subarachnoid hemorrhagic infiltration. On the neck, there were hemorrhagic infiltrations of the injuries that were arranged in a collar (also documented on histological examination) with hemorrhagic infiltration of the epiglottis and hemorrhagic areas of the carotid walls. The lungs were also emphysematous on histological examination with no signs of drowning. Inside the trachea, at the level of the main bronchi, the presence of food material was detected.

In the forensic analysis conducted by the Italian team, the presence of a collar-shaped skin injury at the base of the neck, the presence of bruises on the anterior surface of the left and right arm, and the presence of bruises on the surfaces of the right thigh were attributed to attempts to grasp, which caused (in particular, the neck injury) acute cardiorespiratory insufficiency from violent asphyxiation due to throttling. This postmortem pathological finding was confirmed by histological examination that showed hemorrhagic infiltrations (when the woman was still alive) of the subcutaneous soft tissues and muscles of the neck region as well as pulmonary emphysema. The complete absence of water in the respiratory tract, stomach, and bowel led to the conclusion that death occurred about 30 minutes after the last meal, and the presence of food material inside the trachea and bronchi suggested a regurgitation in the terminal agonic phase. In this case, toxicological investigations were also carried out with an immunoenzymatic method on the ILAB 600 device on blood and urine to search for cannabinoids, opiates, cocaine, amphetamines, methadone, benzodiazepines, barbiturates, tricyclic antidepressants, and ethyl alcohol, which were negative. In the same laboratory, the findings taken by the police on the ship in the couple's room were analyzed, which were identified as fatty acids, vitamin E, acetylsalicylic acid, and ibuprofen. After lengthy investigations from 2006, her husband was arrested in 2008, and subsequently the Italian forensic pathologist who carried out the autopsy was called to testify twice, the latter at the end of February 2019, in Los Angeles, Orange County, CA, where the trial was conducted. The trial ended at the end of September 2019 with a sentence of life imprisonment for the accused.

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### Forensic Sciences, Throttling, Micki Kanesaki Case



**E18 Examining the Escalating Rise of Violence Against Law Enforcement by Youth Street Gangs During the COVID-19 Pandemic**

*Cliff Akiyama, MPH, MA\*, Akiyama and Associates, LLC, Philadelphia, PA 19143*

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**WITHDRAWN**

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## E19 Identifying Markings on Fabric Consistent With Being Created by a Cycling Taser® and the Application of Infrared Photography to Locate Suspected Taser® Probe Markings on a Police Officer's Uniform

*John C. Paolucci, BS\*, CSI Experts Inc, Lagrangeville, NY 12540*

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**Learning Overview:** After attending this presentation, attendees will have learned about characteristics of markings left on fabric by a cycling Taser® in the drive stun mode and how they can be distinguished from extraneous marks and defects on a fabric.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by provided scene investigators and laboratory personnel with information that can be useful in analyzing and comparing statements and electronic data to help determine events that transpired in certain incidents where the use of a Taser® is in question.

Attendees will be made aware of some common characteristics found during the examination of markings created when a cycling Taser® X26 comes into contact with a fabric composed of polyester, and how infrared photography can be used to find these markings. This presentation covers a high-profile officer-involved shooting incident, the shooting of Walter Scott by North Charleston police officer Michael Slager. An analysis of the Taser's® download data revealed two more activations of the Taser® than the officer accounted for in statements immediately following the incident.

The officer's final radio transmission prior to the shooting, which is when the officer claimed that the Taser® was taken from him, was followed by two additional activations of the Taser®. An examination of the officer's uniform shirt was determined to be a necessary investigative step to assist in the reconstruction of events that transpired during that incident. It was hypothesized that a Taser® could create unique markings (unique as in class characteristics and not unique to a particular Taser®) when cycling and coming into contact with a fabric.

A garment's composition and history are essential to many types of analyses ; a uniform shirt that had been purchased together with the evidence shirt and worn alternately by the officer with the evidence shirt was obtained for the purpose of creating exemplars of markings left by the probes of a Taser® when used on the garment in the "drive stun" mode. Infrared photography was used to examine areas where the Taser® came into contact with the test garment as the dark blue dye of the uniform shirt obscured any visual examination for possible markings. Numerous applications of the Taser® to the garment were performed and common characteristics were noted. As the Taser® probes are a fixed distance apart, the marks left on the garment were also a similar distance apart. The bottom probe typically left a larger mark than the top probe, which helps to determine the orientation of the muzzle when examining questioned marks suspected to have been created by a Taser®. Another common characteristic was that the dark discoloration created by the cycling Taser® would typically bypass threads on the top of the weave pattern (standard weave in this case).

Many variables were also explored during the testing process to account for the dynamic events that preceded the shooting. For example, stretching the garment when applying the cycling Taser® would cause marks to be closer together when examined after the tension was released. The garment was sprayed with a saline solution to simulate perspiration prior to the application of the cycling Taser®. Research is continuing in this area and this presentation covers only the preliminary experimentation that was initiated to analyze the veracity of statements made by the subject officer and unexplained activations of the Taser® that were discovered when the download data was analyzed.

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### Officer Involved, Taser®, Infrared Photography



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## E20 Biomechanical Assessment of Various Punching Techniques

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**Learning Overview:** After attending this presentation, attendees will understand the injury risks associated with various punching techniques. In addition, attendees will learn the essential factors influencing the intensity of such blows and the basic physical quantities describing the punch efficacy.

**Impact on the Forensic Science Community:** The assessment of violent acts and the resulting injuries is an important competence for forensic medical experts. However, the literature on this subject is often limited to case reports and the basis of the expertise to a large extent subjective. This presentation will impact the forensic science community by providing quantitative data to base assessments of punches and further studies are planned.

**Introduction:** Punches are a common kind of body violence and are assessed on a regular basis in expert witness testimonies. Apart from the correspondence between the asserted assault and the documented injuries, questions are often raised on behalf of the punch intensity and other biomechanical aspects regarding the specific act of violence. There seems to be a general agreement that a strike with the palm of the hand is less than a fist punch, though robust biomechanical data to support this opinion are missing.

Fifty individuals (male and female) participated in the study, and the main anthropometric characteristics (height, weight, handedness, etc.) were recorded. Study participants performed three kinds of punches: with the fist, with the flat of the hand, and so-called “karate-chops.” As a target, a punching pad was fastened onto a KISTLER force plate (10kHz). In the course of the measurement session with each volunteer, three strikes of each kind were performed, both with the dominant and the non-dominant hand. The punch velocity was established by using a high speed Olympus® camera (2kHz). Afterward, the highest impulse (obtained by the integration of the force-time curve) was selected for further analysis. Also, the effective mass of the punches was computed (using the impulse and the punch velocity). Ultimately, the data underwent several statistical tests to identify significant differences between the outcomes of each individual.

Significant differences between the dominant and the non-dominant hand, as well as between male and female volunteers, were ascertained. The Friedman test showed significant differences among the different punch types for all the parameters on a 0.05 significance level. The post-hoc testing, according to the Nemenyi method, revealed that the fist punch parameters differed from the two other punch types in both the dominant and non-dominant hand. A significant difference between the palm strike and the karate chop was determined for the maximum force and the impulse of the dominant hand and for the impulse, velocity, and effective mass of the non-dominant hand. Also, a relationship between body height and impulse, and even more so between body weight and impulse, in all punching types was detected.

The presented data constitute a solid basis for the comparison of the most relevant physical parameters of different punching techniques and improves the understanding of punch and strike dynamics under various circumstances. A large-area contact leads to lower stresses in the affected tissues and thus a lower risk for contact injuries. The same amount of (maximum) force of a strike with the whole palm and finger area contacting the head leads to a significantly lower injury risk compared to a fist punch with the same force amplitude (with force being transmitted almost exclusively through the knuckles of the bases of the long fingers). It is known that the risk of an injury of a punch to a high degree depends on the assailant’s technical skills. It appears that the impact force and thus the injury risk is higher in skilled persons because of them being able to transfer more of the striking arm’s mass into the punch.

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### Biomechanics, Punching, Forensic Medicine

## E21 A Microscopic and Elemental Analysis of Anthropological and Modern Buried Hair Compared to Soil Composition: A Case Study of a Male Child and Adult Female From the Arch Street Project in Pennsylvania

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**Learning Overview:** After attending this presentation, attendees will have learned about the forensic value of buried hair through simulated modern burials and a case study of anthropological remains recovered from the site of the former First Baptist Church of Philadelphia (FBCP) in Pennsylvania.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further contributing to the existing literature on the forensic value of hair as well as the effect of degradation over time and environmental exposure on the microscopic characteristics and elemental composition of hair.

Several hundred human remains were unearthed during a 2016–2017 construction project at 218 Arch Street, site of the former FBCP. Local archaeologists launched a salvage archaeological project to recover and relocate these remains in what became known as the Arch Street Project. The large quantity of hair recovered on the skulls of two of the remains (a male child [G-9] and adult female [G-33]) provided a unique opportunity to conduct a case study of anthropological hair from the FBCP cemetery, analyzing its morphological features and intrinsic chemical composition as compared to the surrounding soil after years underground. The value of buried anthropological hair as well as the effect of different sample preparation procedures on the hair samples (i.e., hair washing procedures to remove exogenous contamination without damaging the hair) was investigated through visualization with light microscopy and chemically using Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES) multi-elemental analyses.

Simulated burials of modern hair provided insight as to the degradative effects of the environment through various burial intervals (from one week to one year across different soil types), the efficacy of the different hair washing procedures, and the prevalence of hair morphologies typically associated with postmortem hair across antemortem hair samples as a result of burial. Root morphologies on plucked hair samples provided by living donors were analyzed under transmitted and polarized light microscopy. Microscopic comparisons between modern hair from the same donor exposed to different burial durations and washing procedures were also conducted showing various degrees of degradation from burial and potential damage or removal of external soil contaminants from washing.

ICP/OES multi-elemental analysis focused on the parts per million (ppm) level of 14 elements, including major and trace elements in hair and soil (Calcium [Ca], Chromium [Cr], Copper [Cu], Iron [Fe], Potassium [K], Magnesium [Mg], Manganese [Mn], Sodium [Na], Nickel [Ni], Phosphorus [P], and Zinc [Zn]) as well as heavy metal toxins (Arsenic [As], Cadmium [Cd], and Lead [Pb]) found at levels below the detection limits (<2ppm) of the method.<sup>1-5</sup> Statistical analysis (*t*-tests and Analysis of Variance [ANOVA]) of the simulated burial remains yielded significant results across experimental conditions for some, but not all, of the elements studied. Washed versus unwashed hair showed significant ( $p < 0.05$ , two-tailed *t*-test) differences for Zn in unburied controls and Ca in buried hair samples as well as significant ( $p < 0.01$ , two-tailed *t*-test) differences in Na, K, Cu, and Zn levels between the burial durations compared to unburied controls. The anthropological hair samples from G-9 (male child) and G-33 (adult female) were chemically distinct from each other (Mg, Na, Ca, Fe, Mn, P, Pb) as well as chemically distinct from the soil (Fe, K, Mg, Na, Mn, Zn). Results indicate that burial has a significant effect on the mineral content of hair and that surrounding soil should be investigated along with hair samples in these cases, but that even after centuries of soil exposure, anthropological hair retains elemental distributions unique to the individual.

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### Anthropology, Hair Analysis, Burial



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## **E22 The National Institute of Justice’s (NIJ’s) Terrestrial Light Detection And Ranging (LiDAR) Scanning Working Group (TLSWG) for Criminal Justice Applications: Progress to Date**

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**Learning Overview:** After attending this presentation, attendees will be apprised of current NIJ-supported efforts to promote more uniform implementation and use of terrestrial LiDAR scanning technology for criminal justice applications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving attendees ability to attain high-quality and scientifically supportable conclusions from scan data by developing consensus-based guidelines for terrestrial LiDAR scanning.

Terrestrial LiDAR scanning (TSL) devices (also known as terrestrial laser scanning devices) acquire complex geometric data that captures a 3D representation of a scene. Use of this technology is increasing in criminal justice applications, such as documenting a crime or crash scene; however, no standardized, vendor-agnostic guidelines are available to help practitioners successfully use TLS from scan capture through storage.

The NIJ’s Forensic Technology Center of Excellence convened a TLSWG for Criminal Applications to address these key challenges in using TLS. The TLSWG consists of federal, state, county, and local forensic practitioners and researchers with extensive backgrounds in crime scene documentation and reconstruction, as well as experience in providing expert testimony on bloodstain pattern analysis and trajectory reconstruction. The working group also has representation from the Crime Scene Subcommittee of the NIST Organization of Scientific Area Committees (OSAC) and NIJ’s Forensic Science Technology Working Group.

The goal of the TLSWG is to develop resources that reflect consensus-based best practices to standardize and improve the use and application of TLS in crime scene documentation and reconstruction. These deliverables will help establish a minimum standard for capture, processing, analysis, visualization, presentation, and storage of TLS data in a forensic context. These resources are intended to promote uniform implementation and use of TLS technology in practice. This will ultimately improve the practitioners’ abilities to attain scientifically supportable conclusions from TLS data, ensure effective quality management procedures, and improve presentation of this information to stakeholders, including law enforcement, investigators, and the courts (e.g., prosecutors and defense attorneys, judges, and juries).

Through virtual meetings, the TLSWG has identified challenges and key community needs for: (1) equipment procurement, calibration, and validation; (2) data capture and relevant training; and (3) data processing, management, and reporting. In this presentation, the group will communicate key insights that they have developed from these collaborative sessions, including progress on a standardized “field calibration check,” guidance on data management, and the value of additional equipment needed for successful usage of TLS.

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### **Terrestrial LiDAR Scanning, Crime Scene, Documentation**

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## E23 Predicting Alternate Light Absorption in Areas of Trauma Based on Skin Color: Not All Wavelengths Are Equal

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**Learning Overview:** After attending this presentation, attendees will be able to identify which alternate light wavelengths and filters increase the probability of detecting absorption in areas of physical trauma based on skin color.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the likelihood of detecting evidence of physical trauma using an alternate light on diverse skin tones.

According to the National Crime Victimization Survey, less than half of all violent victimizations are reported to the police. Research does suggest victims of violence may be more likely to engage in the criminal justice process if their physical injuries are identified and documented. Unfortunately, individuals with dark skin tones are disadvantaged by current practices assessing soft tissue injuries by the naked eye. To overcome this challenge, a national protocol recommends the use of alternate light to improve the visibility of subtle injuries on adults and adolescents. However, there is limited research to show how skin color affects alternate light wavelengths in the detection of cutaneous light absorption originating from trauma.

The purpose of this study was to determine which wavelengths within the Narrow-Band Visible (NBV) and Ultraviolet (UV) spectrums improved detection of light absorption on areas of trauma over time. A randomized controlled trial was designed to prospectively bruise 157 healthy adults using controlled application of a paintball pellet to a randomly selected upper arm. Participant diversity was assured through quota sampling of six skin color categories determined using colorimetric skin color data collected from the lateral right deltoid. Using a cross-over design, the bruised area was examined 21 times over a four-week period using an Alternate Light Source (ALS) and white light in random order. The presence of light absorption was assessed using wavelength peaks within UV (365nm) and NBV (415nm, 450nm, 475nm, 495nm, 515nm, 535nm) spectrums and filters (yellow, orange, red). Multilevel models were used to account for the correlated repeated measures data collected in this study. The probability of detecting light absorption for individual wavelength/filter combination was calculated and estimates were made for each skin color category (area around the bruise).

Across each skin color category, the expected probability of detecting areas of absorption under alternate light wavelengths using 415nm and 450nm with a yellow filter were higher compared to white light or any other tested wavelength (e.g., dark skin: 415nm: 0.90, 95% CI: [0.87-0.93]; white light: 0.81, 95% CI: [0.77-0.85]). UV was limited in its effectiveness to individuals with light skin (e.g., very light skin: 0.93, 95% CI: [0.90-0.95]; dark skin: 0.20, [0.17-0.24]). ALS wavelengths of 415nm and 450nm provide a greater probability of detecting light absorption in areas of trauma across skin tones. With further development and evaluation of evidence-based practice guidelines, ALS is an ideal adjunctive tool to complement the physical assessment of injuries on diverse populations.

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### Alternate Light Source, Bruise, Skin Color

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## E24 Species-Level Forensic Identification of Illegally Traded Endangered Woods Using a Combination of Mass Spectral and Chemometric Techniques

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**Learning Overview:** The goal of this presentation is to introduce attendees to a rapid and efficient technique for the forensic identification of endangered species of woods. Current techniques are suboptimal for field analysis and attendees will be introduced to multiple mass spectral and chemometric techniques that can be used to resolve this issue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a powerful new approach that will enable wildlife forensic science practitioners to effectively detect and identify illegally traded woods and combat illegal logging.

Wildlife trafficking is one of the most lucrative criminal enterprises in the world. It is international in scope and nets billions of dollars annually. One heavily traded wildlife commodity that occupies a large market share is illegal lumber. Illegal logging and related trade occur when timber is harvested, transported, processed, bought, or sold in violation of national or subnational laws. It is estimated that 70% of the timber exports of some countries is illegal. The revenue generated is often used to fund the illegal activities of organized crime networks and terrorism. Traditional techniques for wood species identification include DNA profiling, morphological feature characterization, and stable isotope analysis. These techniques are suboptimal for field analysis, such as at ports of entry into the United States, because of challenges, including their time-consuming and costly nature, and the requirement for specialized expertise to accomplish the identification. Therefore, a method is needed that would permit prompt detection and identification of illegally traded endangered woods. This study demonstrates that species-level identification can be accomplished through the use of species-specific chemical signatures revealed by Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART<sup>®</sup>-HRMS), along with thermal desorption coupled with Gas Chromatography/Mass Spectrometry (GC/MS). These profiles can be subjected to multivariate statistical analysis processing to produce a screening device against which samples encountered by law enforcement agents can be compared for rapid identification of timber and wood products.

To develop this method, multiple samples of different species of wood representing a range of genera were cut into slivers and placed in scintillation vials. The headspace volatiles of each were concentrated onto conditioned Solid Phase Microextraction (SPME) fibers for 30 minutes. The fibers were then analyzed using DART<sup>®</sup>-HRMS by placing the fiber directly in the DART<sup>®</sup> gas stream for thirty seconds. The wood slivers were also analyzed directly by suspending the wood slivers directly in the DART<sup>®</sup> gas stream. Both sets of results were subjected to multivariate statistical analysis processing, which revealed that chemical fingerprints could be generated from the direct analysis of the bulk material as well as the headspace of wood samples. Successful models for species-level identification of wood samples that featured the data acquired from the application of both techniques were created. Feature masses were selected for each of the data sets, then Kernel Discriminant Analysis (KDA) was used for the statistical analysis processing. The results showed clustering of like species in both of the models. Leave-One-Out Cross-Validation (LOOCV) was used to test the headspace analysis model and showed 97.14% accuracy. External validation was used to test the bulk analysis model and showed 87.50 % accuracy. To identify compounds associated with diagnostic molecular markers (i.e. *m/z* values), slivers of wood from each sample were placed in thermal desorption tubes and their headspace was then analyzed by GC/MS. The observed mass spectral fragmentation patterns not only enabled identification of several of the diagnostic molecular markers, but also revealed that the spectra exhibited interspecies differences and intraspecies similarities. The results from this study indicate proof-of-concept that the mass spectral analysis of wood samples, both headspace and direct, in conjunction with chemometric techniques, can be used to create a database that can be made available to law enforcement agents for the identification of illegally traded wood species.

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### Endangered Wood, Species Identification, Mass Spectrometry

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## E25 Seeing vs. Knowing: Overcoming the Brain's Filters to Render Realistic Forensic Sketches

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how a properly trained forensic artist must develop new interpretive perceptions on how they view human faces in addition to learning facial anatomy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic art can benefit from the artist who has learned how to interpret visual references through light, shadow, and negative spaces rather than trying to render an image modeled from one's own memory and notions of what an image *should* look like. The value of this presentation will be to aid in understanding the obstacles forensic artists face and the reward awareness can impart for better, more realistic composite sketches.

Our brains compile a data set of stock images to which it will refer with regularity based upon what we have seen and interpreted during our lifetimes. These images are compiled from many different formats (moving images, photographs, illustrations, and real subjects). In addition, the human brain wishes to simplify what it cannot recall in detail. Forensic artists must re-train themselves on how to view images as defined by light, shadow, and shape as presented, not as we tend to think of them in 3D space. Filters created by the brain for stored imagery dictate how people or faces are *supposed* to look in our minds. Ironically, these filters often interfere with our ability to generate realistic composite images. A forensic artist's re-training in their perception is necessary in order to bypass the filters our brain imposes. Composite artists must first "unlearn" those filters to generate realistic images from witness descriptions and reference materials.<sup>1-3</sup>

This presentation discusses the skills and training methods utilized by some forensic artists to produce a more realistic composite sketch. The goal of a composite sketch is not to produce portraiture but is about producing a likeness that will aid law enforcement in the recognition and/or identification of a suspect. The composite sketch helps law enforcement eliminate unnecessary time and energy expended toward individuals who do not fit within that suspect pool as described. Digitally generated images and software programs are not the focus of this presentation, but there will be some reference to them as a point of comparison on digitally produced images versus hand-drawn images from witness accounts and ways the brain is or is not engaged in what is produced. This presentation will impact the forensic community by demonstrating how more realistic composite images can be produced with proper training and that such skill is not necessarily rooted only in one's inherent talent.

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### Forensic Art, Brain Patterning, Drawing

## E26 Biological Evidence in Sexual Violence Cases

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of biological evidence in the sexual violence against women and children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the importance of biological evidence in sexual abuse cases and all dimensions of the forensic aspects. Biological evidence is sometimes the only way to demonstrate that sexual contact occurred and to identify the perpetrator.

Sexual crimes involve a sexual purpose committed without the consent of the victim (mostly women, but also children and men) that cause many short- and long-term health problems.<sup>1,2</sup> Biological evidence collected for DNA studies is considered the most important legal evidence due to its usefulness in determining the existence of sexual contact and the identities of suspects. Biological evidence is sometimes the only way to demonstrate that sexual contact occurred and to identify the perpetrator.<sup>3</sup> According to Adam's criteria, one of two criteria that allows a diagnosis of sexual abuse is the presence of semen in samples taken from the victim's body.<sup>3-5</sup> This study examined cases that applied with a claim of sexual violence with the aim of demonstrating the importance of a timely and high-quality forensic medical examination.

In this study, the consents of 61 victims who were referred to Adana Çukurova University School of Medicine Forensic Medicine Department by judiciary authorities between January 1, 2017, and December 31, 2019, as sexual abuse or assault cases within the first seven days of the alleged incident. The study data was computerized and evaluated with Statistical Package for the Social Sciences (SPSS); 41.0% ( $n=25$ ) of the examination of the results of swabs returned positive DNA results. In terms of swab results and application times, the rate of positive results was significantly different in the first 24 hours ( $p=0.016$ ). It was reported that the ratio of DNA obtained from vaginal swabs was 35% on the first day, 26% on the second, and 23% on the third day, and significantly decreased after the third day. DNA was identified in 22% of anal swabs and 41% of skin swabs taken in the first 24 hours, and this ratio decreased to 5% in anal swabs and 14% in skin swabs taken at 24–48 hours.<sup>6</sup>

Clothes were collected from 34.4% ( $n=21$ ) of cases for examination; 61.9% ( $n=13$ ) of these samples collected, such as clothing and underwear, after abuse returned positive results. In terms of clothing examination results and application times, there was no statistically significant difference between groups ( $p > 0.05$ ). Dry secretions on clothing were quite stable, allowing semen to be detected for longer than one year. It is reported that sperm can be detected in cotton fabric or paper samples that are dried before storage.<sup>7</sup>

The timing of the examination in sexual violence cases, especially in cases that require acute intervention, should be set based on the patient's convenience. However, in order not to lose therapeutic opportunities such as emergency contraception, to avoid changes in physical evidence such as injuries, not to lose forensic material that may be used as evidence of contact by the perpetrator, including blood and semen, access to medical services should not be delayed and should be rapidly carried out.<sup>8</sup> Studies confirm that in cases where the examination of prepubertal children is carried out within the first 24 hours after the assault, DNA can be isolated in most cases.<sup>4,9,10</sup>

It was reported that the half-life of sperm in the vaginal cavity depends on the victim's age, and in cases where sperm is localized in the cervix, its half-life may be more than 72 hours. In post-pubertal females, it is reported that spermatozoa can remain mobile for 6 to 12 hours in vaginal secretions and 5 days in the cervix, while in pre-pubertal female, the half-life of semen is shorter due to the lack of cervical mucus.<sup>3,7</sup> It is noted that the reported half-lives generally indicate estimates, and variables such as the victim's gender, age, activities after sexual contact (such as urinating, excreting, vomiting, brushing teeth, having a bath, eating, drinking, smoking, spitting, running, and walking), condom use, and azoospermic perpetrators will also influence the results.<sup>3</sup>

A high-quality forensic medical examination may potentially confirm and/or relieve the sexual assault victims' concerns, minimize their trauma, and support their recovery. Additionally, the collected evidence may help reveal the material truth during the criminal investigation, leading to the execution of criminal sentences and the prevention of further sexual violence.<sup>11</sup>

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## Biological Evidence, Sexual Violence, Forensic Genetics



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## E27 Forensic Science Learning for Victims of Enforced Disappearances: Study Results

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**Learning Overview:** After attending this presentation, attendees will have learned the results of an innovative teaching program in basic forensic science and crime scene investigation developed for the relatives of victims of enforced disappearances and violations of human rights.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that people without any background in/knowledge of forensic science and crime scene investigation field are able to address and process a crime scene.

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 the world’s history. Some of the most remarkable contexts are dictatorships, armed conflicts, and drug trafficking. These illegal actions have been used as a terror-spreading strategy in several 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 victims themselves and governments but also for their close relatives.

Drug trafficking is considered one of the most important contexts in which numerous people are being declared “missing,” and Mexico ranks among the countries with the highest number of enforced disappearances. This is why many groups of close relatives of enforced disappeared people have been formed throughout Mexico, initially with the main goal of supporting the close relatives of the “missing,” but subsequently with the purpose of going out into the field searching for any kind of physical evidence that can be provided to the prosecutor’s office and help with the investigation of these cases. One these groups is Sabuesos Guerreras (Warrior Hounds), a not-for-profit organization from the city of Culiacan, in state of Sinaloa, Mexico. This group is composed mainly of women whose beloved husbands and/or sons have been victims of enforced disappearances, and for years, besides supporting each other sociologically and economically, they have searched for physical evidence in different open fields of the state.

The aim for cooperating in the world’s fight for human rights as well as helping groups like the Sabuesos Guerreras is the inspiration for creating an innovative training program in basic forensic science with the main goal for these groups to be able to use the knowledge they are acquiring through this program in their own relatives’ cases. This training program is a ten-class online course, one lesson per week, with three main learning subjects: 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 very same evaluation instrument. This assessment consists of four questions relative to the subjects of the course, and their goal will be assessing the amount of learning acquired throughout the course by the participants. Also, the participants will know the results of the searching process at the field after the students have finished this course. This program was created as a result of a PhD thesis and has been developed to be offered online from Chaminade University of Honolulu, HI, to its recipients in Culiacan, Sinaloa State, Mexico, in Spanish. This educational program can be utilized for other groups of victims of enforced disappearances around the world in different languages.

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### Enforced Disappearances Victims, Online Teaching Program, Education

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## E28 Military Forensic Science—From the Battlefield to the Courtroom

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**Learning Overview:** After attending this presentation, attendees will understand the principals of military battlefield forensic science and the practical application of military forensic science at level 1 (collection), level 2 (deployable laboratory), and level 3 (national laboratory facilities).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the importance of a global forensic science under a unified system.

The changing nature of war in relation to global terrorism, foreign terrorist fighters, and length of conflict has resulted in a transformation of the strategic environment from previous conventional warfare. This has contributed to the blurring of the lines between military objectives and law enforcement. Forensic science has been employed beyond its traditional support of law enforcement investigations for the criminal justice system to also supporting military operations. Historically, forensic science in the military domain matured during the Iraq and Afghanistan conflicts when coalition partners were faced by an Improvised Explosive Device (IED) threat that required identification of threat actors. Terrorism does not respect international borders and IEDs collected on the battlefield can be used as evidence to prosecute returned foreign fighters in national criminal proceedings. This requires a unified approach to forensic science that integrates military operations with law enforcement investigations. In a unified approach to forensic science, military forensic science is conducted under a quality management system to ensure that the material collected and examined is done so in a forensically sound manner. This is to ensure support can be provided to forensic intelligence objects on the battlefield or rule of law objectives to support criminal prosecutions, if required.

Case studies have demonstrated the importance of applying a unified approach to forensic science. This presentation will outline the case study of the Crown prosecution of a British citizen for the extraterritorial murder of Sergeant First Class (SFC) Randy Johnson from the United States 2<sup>nd</sup> Cavalry Regiment. In 2007, SFC Johnson was murdered in an IED attack in Iraq. The IED fragments were analyzed at the Federal Bureau of Investigation (FBI), Terrorist Explosive Device Analytical Center (TEDAC) level 3 facility. In 2014, fingerprints and toolmarks from four devices were recovered from IEDs that were linked to a bomb-making cell that was operating in Iraq that involved a British citizen, Anis Abid Sardar. In 2007, Sardar travelled back to London from Iraq where he was stopped by officers at Heathrow Airport, and fingerprints and DNA samples were taken. The United Kingdom authorities had shared Sardar's fingerprints with the United States as a known or suspected terrorist file and thus a match between the fingermarks on the IED collected on the battlefield and the fingerprints collected at the UK border was able to be made. On September 23, Sardar was arrested in his home in London for the murder of SFC Johnston and prosecuted by the Crown Prosecution Service. Sardar was sentenced to 35 years for the murder of SFC Johnston.

The case study of the prosecution of Sardar by the Crown Prosecution Service demonstrates the importance of a unified model for forensic science as operations that originate on the battlefield could transition into criminal prosecutions. There is a need to ensure that military forensic science is conducted under appropriate quality management systems so that timely forensic intelligence objectives are met, but the forensic integrity is maintained. This presentation aims to increase the awareness of the unique aspects of military forensic science, which are often overlooked by traditional forensic science practitioners.

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### Military, Foreign Terrorist Fighters, Improvised Explosive Device



## **E29 Utilizing the National Missing and Unidentified Persons System (NamUs) to Resolve Missing, Unidentified, and Unclaimed Person Cases**

*Bobbie B.J. Spamer, MS\*, National Missing and Unidentified Persons System (NamUs), Fort Worth, TX 76107*

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**WITHDRAWN**

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## E30 Spatial and Demographic Patterns of Resolved Missing Persons Cases: What the National Missing and Unidentified Persons System (NamUs) Data Tell Us

*Ann W. Bunch, PhD\*, State University of New York Brockport, Brockport, NY 14420*

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**Learning Overview:** The goal of this presentation is to demonstrate patterns found in a national database (NamUs) regarding spatial and demographic variables in resolved cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing questions regarding resolved missing person cases in the United States as a follow-up to a smaller 2013 county-based study.

NamUs has been used effectively by Medical Examiner's Offices (MEOs) in the United States since its inception in 2005. The investigative tool allows for the collection of important digital information from the public and from investigative agencies in order to develop profiles of missing persons and, at the same time, the compilation of biological and other scientific data of unidentified remains currently held by MEOs. Having these two separate databases has permitted death investigators, medical examiners, and other investigative personnel to run comparisons between them in order to determine if consistencies exist. If such similarities are found, follow-up testing and comparisons are conducted. The use of NamUs databases has resulted in 883 identifications by 2018. There is no doubt that NamUs has been an important positive development in the resolution of missing person cases in the United States.

These two databases were merged for the purposes of this study in order to analyze resolved (e.g., identified) cases ( $N=883$ ), especially with an eye toward spatial factors. Bunch, Kim, and Brunelli conducted a county-wide study utilizing Geographic Information Systems (GIS) and investigator case notes in order to determine if any spatial patterning existed between the Victim Last Seen (VLS) location and the victim Body Recovered (BR) location.<sup>1</sup> Intriguing patterns did emerge and indicated that further study was warranted. The NamUs database records location of BR and, at times, VLS by county so that precision is limited in most cases. However, analysis using the county designations can be generally informative of relative proximity between these two data points. Within the merged NamUs data, the following spatial patterns emerged: 60% of remains were found (BR) within the borders of the same county where the missing person was last known to be alive. Moving further away from the VLS location, NamUs data showed that 17% of remains were found (BR) in the county adjacent to where the missing person was last known to be alive. Thus, 77% of resolved cases were those recovered (BR) within the same county as VLS (59%) or within the borders of the adjacent county to VLS (17%). Along with proximity patterns, this analysis will consider regions within the United States in order to understand where people are reported missing and where those cases are more likely to be resolved.

Demographic patterns were also explored, including sex, ancestry and age. Overall, 585 of the 883 cases, or 66%, were male. Males are overrepresented in a statistically significant way in the resolved missing person population based on this reported data because, according to the 2010 census, males make up 49.1% of the United States population. ( $\chi^2=5.913$ ,  $p$  value=.015029, significant at  $p < .05$  level). With regard to ancestry (or bioaffinity), compared with 2010 census figures, the NamUs unidentified missing person and resolved cases roughly reflect the demographics of the country overall, with Blacks being the only group that is overrepresented in the missing and resolved cases, yet not in a statistically significant way. Analysis of age of those who have been identified via NamUs show the very young (0–17) and the elderly (65+) as the least numerous categories with 56 (or 6%) and 98 (or 11%), respectively. Adults from 18–24 years (110, or 12%), 25–44 years (354, or 40%), and those from 45–64 years (260, or 29%) comprised the vast majority of cases.

Additional spatial/demographic factors will be considered, and some interpretation of the observed patterns will be provided. The use of these data gives us a better understanding of the missing person reporting, investigatory, and recovery process in the United States; it can also be employed as a helpful and applicable tool when investigators approach "cold" or new missing person cases.

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### Missing Persons, NaMUS, Death Investigation

## E31 Using Computer Measurements to Improve Evaluator Reliability of Credibility Assessment With Human Physiology Waveforms

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**Learning Overview:** After attending this presentation, attendees will understand how using computer measurements of human physiology can aid in evaluator decision-making using an analytical theory for credibility assessment. Historically, evaluators utilized visual identification of pattern analysis in human physiology waveforms to make determinations of truth or deception. This presentation will show how combining objective computer measures with data quality assessments can improve the reliability of decisions made by evaluators.

**Impact on the Forensic Science Community:** Credibility assessment is now 100 years old yet suffers from a mixed reputation of acceptance. This presentation will impact the forensic science community by increasing openness to embracing the potential accuracy of credibility assessment when data quality standards are utilized and evaluator objectivity is increased for decisions with computer measurement tools. Previously, credibility assessment standards have not provided clear data quality standards and have not incorporated computer measurements of data to aid evaluators in decision making.

In polygraphy, the Comparison Question Technique (CQT) is used to contrast physiological arousal during multiple presentations of relevant issue questions with comparison questions. Evaluators award diagnostic points at each relevant question and physiology component for an aggregate numerical test score. Exceeding recommended cut scores allows for test decisions. Previous studies have used small numbers of evaluators to analyze large numbers of exams to demonstrate efficacy of this methodology. Previous studies have demonstrated accuracy levels better than chance, yet also demonstrate a higher-than-expected Standard Deviation (SD) for aggregate test scores. It is unknown if this SD derives from differences in test data quality or from the evaluators in reliably assessing decision points during a test evaluation.

In this study, more than 100 evaluators were used to evaluate three field polygraph exams with confirmed results to better understand SD. Each field exam was a single issue with 2 relevant questions and 18 decision points that aggregated for a test score. This study treated each decision point as 54 unique decisions from the three exams to examine evaluator concurrence. Examiner concurrence is defined as at least 90% of the evaluators.

A Data Quality Coefficient (DQC) was also created for each of the 54 decision points, along with a computer measurement of the physiological waveform data collected to contrast with evaluator decisions. This method should identify if computer measures would aid evaluators in their decision-making, thus increasing inter-rater reliability. Further, this study should aid in examining the extent to which data quality contributes to SD and variance.

There were three confirmed polygraph examinations used for this study: one with a high aggregate decision score for deception, one with a moderate aggregate decision score for truthfulness, and one with an exceptionally low aggregate decision score for an inconclusive result. For the two conclusive exams, the evaluators concurred on the correct analysis using visual analytics. However, the evaluators only concurred on 14 of the 54 decision points contained in the three polygraph exams. As well, on the inconclusive exam, approximately 20% of the evaluators incorrectly made conclusive opinions of either truth or deception opinions on the low data quality exam. There was a direct correlation to examiners awarding diagnostic points at those decision points with high DQCs, with a reduction in diagnostic decision points on moderate and low DQCs. Most evaluators agree the easiest waveform for analysis is the Electrodermal Activity (EDA) component where this study had the highest evaluator concurrence at 68.6%. Arguably, the most complex component for visual analysis is the respiratory waveform. In this study, respiration had the lowest evaluator concurrence at 44.7%. The use of computer measurements of the respiratory component alone would have improved evaluator concurrence by 55.5% of those decision points and reduced several evaluator errors.

This study supports the idea that evaluators would benefit from data quality standards in making decisions. Currently, the complexity of assessing data quality is still uniquely best handled by human evaluators. However, this study clearly suggests evaluators would improve their reliability by using objective computer measurements to augment decision-making where computers demonstrate greater objectivity.

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**Pattern Analysis, Decision Reliability, Credibility Assessment**

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### E32 Dating Apps and Their Implications on Child Sexual Abuse: A Discussion of One Such Case

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**Learning Overview:** After attending this presentation, attendees will be aware of the various issues that arise in cases of apps and online dating profiles and child sexual abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the socio-demographic profile of the perpetrators as well as the social and legal implications when dealing with child sex abuse cases.

With the advances in technology, children and young people can communicate freely and are in touch with people with whom they would otherwise not interact. Children and young people who are involved in internet-related child sexual abuse and exploitation are unlikely to tell someone that they have been harmed. They are often discovered accidentally by parents. The ease of communication, without barriers or monitoring, has enabled not only positive interactions but negative ones as well. There is also a lack of inhibition in young peoples' online behavior as a contributing factor and the dangers of misinterpretations of facts and personal information by either party.

For the scattered Lesbian, Gay, Bisexual, Transgender and Queer or Questioning (LGBTQ) community, the best bet to find likeminded people is on the internet. Hence, the third most common group that is at risk are young people exploring their sexual orientation seeking appropriate relationships but not having a healthy or positive way to do so offline who go online for acceptance and find themselves caught up with perpetrators. An annual survey reveals that young males are now using online dating apps to explore their sexuality and meet local men who are willing to engage in sexual activities. The darker side is far from the rosy idea of finding a perfect match.

Section 377 was struck down by the Supreme Court of India on September 6 to make all types of consensual sex between "adults" legal. Protection of Children from Sexual Offences (POCSO) 2012 protects children against all types of sexual abuse. Children of all genders are protected under this act. Under POCSO and Section 377, the adult is liable for prosecution. Under POCSO, even sending nude pictures to minors and encouraging minors to send nude pictures is a crime. Physical contact is not required.

The safeguarding of children and young people brings with it new considerations of children's rights, such as right to privacy alongside the right to protection. The present case discusses one such disturbing incidence and the various measures which can be taken to combat such issues. It also discusses the responsibility and accountability of members and of dating platforms to verify the information of its members to restrict children and juveniles from access.

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#### Dating Apps, Child Sexual Abuse, Child Rights



**E33 Officer-Involved Shootings (OIS) ... Premature Conclusions**

*Rod Englert, BS\*, Englert Forensic Consultants, West Linn, OR 97068*

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**WITHDRAWN**

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**E34 A Vapochromic Colorimetric Sensor for the Cross-Contamination of Volatile Organic Compounds (VOCs)**

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**Learning Overview:** After attending this presentation, attendees will be more aware of the developments with novel colorimetric sensors that can be used to determine cross-contamination of VOCs that may occur through canine training aid storage.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel, inexpensive, field-portable vapor sensor that will allow for the improved storage and implementation of training aids, resulting in increased efficiency and reliability of canine teams.

Similar chemicals are often stored together in enclosed spaces with little thought to possible cross-contamination. In most cases, the contamination of trace levels of VOCs is not a significant cause for concern. However, it is relevant in the case of canine scent training, as a canine's superior sense of smell is very likely to detect this contamination, even at trace levels, while humans often remain unaware of the contamination, contributing to inefficient training. Canine training aid kits contain multiple chemicals stored at a variety of temperatures and conditions that could lead to cross-contamination of co-located training aids, and subsequent use of these aids may eventually result in less reliable canine units. Thus, the need for a simple, field-portable, vapochromic sensor to determine the cross-contamination of VOCs within canine training aid kits is addressed.

The development of a vapor sensor is proposed, which produces a rapid colorimetric change when in the presence of certain volatile chemicals. Solvatochromic compounds, which change color when dissolved in solvents with different polarities, are used as indicators and deposited onto a cellulose substrate. This substrate was chosen due to its wide availability, biodegradability, low cost, and ease of use and disposal. The sensors' solvatochromic properties are then translated to vapochromic capabilities by exposing it to the vapors of the analyte of interest, instead of directly interacting with a solvent. The indicator reacts with a VOC that is similar in vapor pressure to the most volatile compound inside the canine training kit of interest to produce a rapid change in color visible to the naked eye. To increase vapochromic response and device sensitivity, the solvatochromic compound can be incorporated into an adsorbent matrix that would allow the VOC to condense and concentrate near the indicator. This design will allow the sensor to present a comprehensible and unambiguous visible response to the release of VOCs within a closed container, which is indicative of possible cross-contamination of adjacent canine training aids. It can be readily incorporated into existing training kits and will function as a straightforward reminder of when training aids need to be changed or a new containment system should be considered. Moreover, this sensor has the potential to be implemented in other enclosed spaces with the need to determine cross-contamination, such as food storage areas, industrial storage units, and law enforcement evidence lockers.

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**Colorimetric Sensors, VOC Cross-Contamination, Solvatochromic/Vapochromic**



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### E35 Different Chemical Processes on Various Types of Tape to Visualize Latent Prints

*Diana Martinez, MFS\*, National University, La Jolla, CA 92037; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding that not all chemical processes to visualize latent prints will yield the same results on various types of tapes. A process that produces a clear latent print on one type of tape will not produce the same result on another.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping laboratory technicians determine which process would be the best choice for developing latent fingerprint evidence. Once a process has been used, it is irreversible and there can be no further development on the evidence item. Determining the correct process that will allow for further testing while producing the best results can aid in the development of additional latent prints. This can strengthen a case or outcome in court and exonerate an innocent person.

Tape is a widely used tool in crimes. It is mostly used for gagging or as a ligature, but there are other uses for it. It can be used to package drugs, in disposing of a body, and for explosive devices. There can be significant trace evidence found on tape: hairs or fibers, touch DNA from handling the tape, and even latent fingerprints. These can be recovered from both the adhesive and non-adhesive sides of the tape. With various types of tape available, there are also various processes to recover identifiable latent prints. This study determined which of several widely used chemical development processes produced the best results on the adhesive side of various widely used tapes, including electrical, blue painter's, packaging, and duct tapes.

Lengths of tapes were cut from unopened rolls and were processed with ten different chemical developers after a male and female volunteer were instructed on how to deposit their fingerprint (thumb or index finger) on the adhesive side of tapes. The developing process included: cyanoacrylate fuming, adhesive side powder, crystal violet, black and white wet powder, Methylene Blue Dye (MBD), and M-Star. All samples were first processed with cyanoacrylate fuming before moving on to the next process.

A total of 80 samples were processed, and the visible latent prints analyzed. For the 40 male samples, only 5 samples produced Level 3 detail. For the female samples, there were 13 prints with Level 3 detail. Male samples had 4 prints with Level 2 detail, female samples had 15 prints. Male samples had 11 prints with Level 1 detail, female samples had 7 prints. Male samples had 20 prints with no value, females had 5 prints. Female samples produced the highest Level 3 and Level 2 prints, making them better producers of ridge detail than male. MBD had an average of no value for male prints; female prints had an average of Level 2 detail.

For male prints, duct tape processed with adhesive-side powder produced the best results, Level 3 detail. Packaging tape processed with adhesive-side powder, crystal violet, white wet powder, and M-star with crystal violet produced Level 3 detail. Blue painter's tape processed with white wet powder produced Level 1 detail; all other processes produced nothing of value. Electrical tape processed with white wet powder produced Level 2 detail, which was the best result produced.

For female prints, duct tape processed with adhesive-side powder, MBD, MBD with crystal violet, M-Star with black wet powder, and M-Star with crystal violet produced Level 3 detail. Packaging tape processed with adhesive side powder, crystal violet, black and white wet powder, and MBD with crystal violet produced Level 3 results. Blue painter's tape processed with black wet powder produced Level 3 results. Electrical tape processed with adhesive side powder and white wet powder produced Level 3 results.

Female prints overall produced the best level of detail across all samples. Blue painter's tape had the least amount of detail for both male and female prints, while packaging tape had the best. Adhesive-side powder produced the best level of detail on both male and female samples.

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#### Latent Fingerprints, Tape Evidence, Cyanoacrylate Fuming

## E36 The Enhancement of Tattoos Using Alternative Light Sources vs. Photo Editing Software

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**Learning Overview:** After attending this presentation, attendees will have a better idea of how alternative light sources and digital photo editing software are used to enhance tattoos that were not clearly visible with the naked eye.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating enhancement techniques in photography that can help visualize tattoos that are obscured due to postmortem changes.

In addition to using conventional photography equipment and techniques, modified cameras and specialized filters can be used to capture obscured identifying features such as tattoos for unidentified decedents. Tattoos are especially helpful when fingerprints or other identifying features are not able to be captured. Even if tattoos are obscured to the naked eye due to postmortem changes, they can often be photographically documented with the use of enhancement methods such as alternative light sources or digital imaging software. Alternative light source photography is a technique that uses infrared and ultraviolet wavelengths from just outside the visible light spectrum to capture what our eyes are unable to see. Additionally, digital imaging software such as Adobe® Photoshop® offers tools that can bring out details that are otherwise difficult to see. Throughout this presentation, these methods will be explored to demonstrate the utility of each in bringing out tattoos on charred and decomposed decedents.

In 2018, it was reported that nearly 46% of the United States population has at least one tattoo.<sup>1</sup> Tattoos are typically highly personalized and even similar tattoos can be distinguished by ink color, size, and body location. When common identification methods like facial ID or fingerprints become a challenge due to trauma or postmortem changes, tattoos can remain a viable option with the help of alternative light sources and/or photo editing software. The most useful wavelengths for uncovering tattoos are near-infrared, which is between 700 to 900 nanometers in the electromagnetic spectrum. These wavelengths help visualize details beneath the epidermis. When a tattoo is created, the needle carrying the ink goes through the epidermis and into the dermis layer of the skin. As the ink stabilizes in the dermis and the epidermis heals, the tattoo becomes a permanent mark in the body. Digital photo editing software may also be utilized to bring out these obscured features. By using a conventional image of the tattoo and modifying the color channels, details that were not clearly visible can be enhanced. By utilizing these techniques, these identifying marks can become a useful tool in forensic identification and photographic documentation.

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### Forensic Photography, Alternative Light Source, Tattoo

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**E37 Evaluating the Effects of Heat on DNA Recovered From Friction Ridges Developed With 1,2-Indanedione (IND)**

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the effect of heat on obtaining usable DNA from friction ridges following latent print processing. IND used to develop friction ridges on paper requires the application of high heat.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a greater understanding that using IND and heat to develop friction ridges on paper has no effect on subsequent DNA analysis. As a result of this testing, the forensic science community can benefit from knowing that evidence treated with IND can safely proceed from a friction ridge unit into a DNA unit for further analysis without hindrance.

**Introduction:** The development of friction ridge impressions on porous items, such as paper, is typically very successful because available techniques target the residues that rapidly absorb into the fibers of the paper, thereby protecting the impression from environmental interference. In addition to the friction ridge residue constituents, DNA is similarly co-deposited when paper is touched or handled. As potentially probative evidence, developing an identifiable fingerprint and DNA profile from the same piece of paper could be valuable, but it must be tested to ensure congruence among both forensic testing analyses. For downstream DNA analysis to be successful, the selected fingerprint development technique must not impede the upcoming biological testing. Current research suggests that a number of chemical enhancement methods, including IND, do not affect subsequent DNA testing, particularly with regard to fingerprints left in saliva or blood. However, there is little research available on the effects of heat in conjunction with IND on cell-free DNA deposits, or “touch” DNA. That is what was tested by this study.

Three fingerprints from 12 participants were deposited onto standard copy paper and the fingerprints were cut in half. Due to the variability of DNA present on friction ridges not only from person to person, but also from mark to mark, each fingerprint was compared to itself. One of the fingerprint halves for each of the three sets was left untreated to compare with the corresponding fingerprint half treated with IND and heat. The three treated fingerprint halves for each participant were subjected to the following heat tests: (1) IND reagent and no heat, (2) IND reagent and moderate heat (60°C), and (3) IND reagent and high heat (100°C). Following visualization and analysis of fingerprint value, the samples, both treated and non-treated (72 total), were cut into pieces and analyzed for DNA five days after being deposited.

Nine of the participants left enough DNA with the fingermarks to move past quantitation for Short Tandem Repeat (STR) analysis. These same participant fingerprints also moved past quantitation following IND and heat testing, having both high quantity and quality of DNA. There were no indications of DNA degradation due to the treatment. Three of the participants, however, initially left little-to-no DNA with their fingerprints, but the friction ridges, upon visualization, were high-quality marks. By looking at the developed print, there was no way to indicate whether DNA was co-deposited, because all fingerprints had high-quality, robust features after being processed with IND and heat.

Overall, it can be understood from this research that there is no significant impact on the quantity or quality of DNA following fingerprint development with IND and heat. Further research can be conducted to determine if there is an impact on DNA analysis after more than five days.

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**Indanedione, Latent Print Friction Ridges, DNA**

## E38 Forensic Analysis by Raman Spectroscopy

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**Learning Overview:** After attending this presentation; attendees will better understand the use of Raman spectroscopy to examine wide-ranging evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering new approaches for the fields of using Raman spectroscopy and for improving existing studies.

Raman spectroscopy is a valuable analysis method for not getting fluorescence from water-containing samples, having the possibility of non-destructive analysis as well as the ability to analyze solids, liquids and gases. There are various types of evidence, which are categorized as biological (DNA, body fluids, bone), physical-chemical (chemicals, explosives, dyes, cosmetic products, fiber, hair, gunshot residue, documents, environmental pollutants), and trace (fingerprint) evidence, in this study to examine using Raman spectroscopy. Wang et al. revealed the difference between human and non-human blood using Raman spectroscopy; results showed that the model provided 100% accuracy in its differentiation between human and non-human blood.<sup>1</sup> In a study that may play a critical role, particularly in rape cases, the separation of peripheral blood and menstrual blood was revealed by Sikirzhyskaya et al. in 2012 with Near-Infrared (NIR) Raman spectroscopy.<sup>2</sup> A condom-related DNA study by Raman spectroscopy was published in 2008 by Coyle et al.<sup>3</sup> In 2016, Delannoy et al. buried bone samples in the soil and included Raman spectroscopy into their work to measure bones' weight every day, over a three-month period.<sup>4</sup> As a result, they reported a decrease in bone mass that was determined by Raman spectroscopy over time. Kwok et al. determined the difference in the packaging of original and counterfeit samples of a drug using Raman spectroscopy and 2D Correlation Spectroscopy (2D CoS).<sup>5</sup> Edwards et al. used Raman Spectroscopy to examine dyes on a painting that was purported to belong to the Renaissance period painter Malatesta.<sup>6</sup> As a result, the pigments of dye that were found were compatible with the Renaissance period. Bianchi et al., in a study conducted with cotton, polyester, and polyamide fabric samples, revealed that the difference between fibers can be determined both in terms of dye and in terms of age.<sup>7</sup>

In addition, the development of portable Raman devices allows evidence to be quickly identified and analyzed during crime scene investigations. In this way, Raman spectroscopy allows the crime scene to be turned into a laboratory environment, which helps analysts and researchers in terms of safety and time. Pestle et al., in their study with the portable Raman spectrophotometer, developed a method to test the presence of sufficient collagen in bone samples found in the field.<sup>8</sup> This method is very valuable for forensic archaeologists as it provides a preliminary study of which sample is worth testing to obtain DNA.

It should not be ignored that Raman spectroscopy alone may be insufficient in evaluating evidence and giving information about victims, suspects, or objects, so it should be verified with an advanced technique. However, modified sample preparation steps and systems such as Surface-Enhanced Raman Spectroscopy (SERS) continue developing to remove these disadvantages in standard Raman analyses. Yu and White, who worked with SERS for the analysis of cocaine and heroin at lower concentrations, developed a paper swab in which they inkjet impregnated silver nanoparticles for sampling.<sup>9</sup> Fierro-Mercado and Hernandez-Rivera developed a substrate by spraying gold nanoparticles on a standard filter paper and reported that this swab could be used to take samples from the contact surfaces of explosives.<sup>10</sup> Becue et al. developed a method, by using a Multi-Metal Deposition (MMD) technique, for the detection of fingerprints from different substrates.<sup>11</sup>

As a result, along with a few disadvantages, standard Raman spectroscopy and SERS have proven to be remarkable and important techniques in the search for more accurate, fast, and portable analysis techniques for modern forensic science. The advantages of Raman spectroscopy over other analytical techniques makes it attractive and inevitable for use in multidisciplinary forensic science. In all aspects, Raman spectroscopy is a valuable universal tool to assist researchers in securing justice.

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## **Raman Spectroscopy, Crime Scene, Forensic Science**

## E39 Textile Biodeterioration: Postmortem Interval (PMI) Estimation With Image Analysis Software in Burial, Surface, and Freshwater Environments

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**Learning Overview:** After attending this presentation, attendees will have a new perspective on the use of textiles at forensic scenes, an understanding of textile biodeterioration rates for common clothing items in specific habitats, and knowledge of proper protocols for documenting forensic textiles and analyzing the images in the software ImageJ.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel, quantitative method for measuring textile biodeterioration with the potential to estimate PMI with clothing found at crime scenes. This presentation also includes vital inter-observer error rates and demonstrates textile biodeterioration rates in the previously unstudied temperate deciduous forest biome.

Estimating the PMI can be challenging, especially in outdoor settings where numerous environmental variables complicate analyses and trends. As time passes, crucial data and context are lost, and the accuracy and precision of physiological changes and entomological indicators are reduced. Because clothing is often deposited at the scene near the time of death, either on the human remains or nearby, the textiles are exposed to the same environmental factors over the same time period. While a few studies have tested textile biodeterioration rates for application to PMI estimation, there is an overall lack of consistency across the types of textiles utilized, the environments tested, and the scoring method employed. Furthermore, the majority of existing literature rely on qualitative observations to estimate the amount of textile degradation with vague, often only descriptive categories. These problems obfuscate any trends that may exist and prevent comparisons between datasets.

The current study aims to address the lack of quantitative data through a novel application of open-source image analysis software to measure the amount of textile biodeterioration over time. It is hypothesized that: (1) the textiles will follow a predictable (linear) pattern of biodeterioration over time; and (2) the biodeterioration rates will be significantly different between settings and between textile types. Five common clothing textiles (100% cotton, denim, linen, 50-50 cotton-polyester blend, and merino wool) were utilized. The five textiles were cut into 10cm by 10cm swatches. These swatches, along with whole garments composed of the same five textiles, were weighed and photographed prior to placement. The samples were placed at The University of Akron's Field Station in Bath, OH. The field station is located on the Bath Nature Preserve, which contains a variety of habitats in the temperate deciduous forest biome. Swatches and one complete garment of each textile type were placed in three different locations: on the ground surface (forested area), buried at a depth of 5cm (forested area), and suspended in a freshwater pond. Three swatches of each textile type were collected from each location every two weeks. The whole garments were collected at the end of the study period. The study spanned the spring, summer, and fall seasons and the samples were exposed to the environment up to 238 days. Upon collection, each sample was dried on a flat surface for 48 hours and then gently cleaned with a soft brush, weighed, and photographed. Swatches were placed in a magnetic Plexiglas® picture frame to ensure all aspects of the samples remained flat during photography. The total surface area of each sample was measured via photos with the software ImageJ before and after placement in the field.<sup>1</sup> The measurements were used to determine the percentage of each textile sample remaining after each sampling interval.

Significant trends were identified using a simple linear regression that modeled the degree of deterioration in relation to the time elapsed. Analysis of each image was conducted by multiple individuals and inter-observer error was calculated. The rate of degradation was fastest in the burial environment and slowest in the freshwater environment. Cotton samples degraded at the greatest rate overall, followed by linen, denim, and wool, with the cotton-polyester blend degrading at the slowest rate. This study offers a novel, simple, and cost-effective method to quantitatively measure textile biodeterioration and estimate PMI with clothing. This data is especially useful, as it provides replicable methods, measures textile biodeterioration rates in a previously unstudied biome, and provides inter-observer error rates.

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### Postmortem Interval, Textiles, Image Analysis

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## E40 12 Myths About Genetic Genealogy

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**Learning Overview:** After attending this presentation, attendees will better understand how genetic genealogy is used to solve crimes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by dispelling 12 common myths regarding genetic genealogy and how it is used to help identify unknown contributors and solve cold cases. This presentation will also help clarify terminology that often is misused and misunderstood in the context of genetic genealogy,

Ever since the breaking of the Golden State Killer case and the arrest of Joseph James DeAngelo in April 2018, the use of genetic genealogy—a relatively new method of crime solving—has become increasingly accepted. The forensic community is finally talking about genetic genealogy (AAFS had a genetic genealogy workshop at the 2019 annual conference in Anaheim, CA), but many do not understand the approach or how much work it entails. Additionally, there is a lot of confusion about the benefits and risks of using genetic genealogy. It is not rocket science, nor is it simply a matter of typing a name into a database and getting a “hit.”

Genetic genealogy has been used for years, but the rise in popularity of consumer DNA tests continues to increase the effectiveness of this approach. Combining autosomal DNA data from at-home DNA tests with traditional genealogical research, one can determine the level and type of genetic and familial relationship between individuals. The increased pool of people in these consumer DNA databases increases the number of DNA matches that can be used for genetic genealogy. This approach is most often used to help identify an adoptee’s likely birth parents or the unknown parent of a child of a Non-Paternity Event (NPE). Since the arrest of the Golden State Killer, more than 100 suspects have been identified this way. Now that more and more law enforcement agencies are learning about the possibilities of using these methods to help solve cold cases, it is important to shed light on how a genetic genealogist does their work ethically and precisely.

This session will address a variety of assumptions people make about how genetic genealogy works and what happens if they “opt in. For example, some people think genetic genealogy works like the combined DNA Index System (CODIS): upload the DNA and the name of the contributor pops out. Other people are afraid if they “opt in,” law enforcement will have access to health data derived from their DNA or can use their DNA to frame them for a crime. At the end of this session, attendees will have more accurate information about genetic genealogy. This presentation will also clarify a variety of terms that are often used incorrectly or interchangeably, such as genetic genealogy, familial searching, investigative genetic genealogy, and forensic genealogy. To benefit the forensic science community, this presentation will touch on the application of genetic genealogy, its legal and ethical implications, its methodology, and the terms that are used to describe it.

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### Forensic, Genetic, Genealogy

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## E41 An Elderly Woman Living With Her Dead Son: A Case Report

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**Learning Overview:** The goals of this presentation are to alert attendees through the use of this case of an elderly woman living with her dead son for six years about the isolation of people and to show the adaptability of forensic pathologists in the forensic investigation of a mummified case,

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the amazing scarcity of the “living with the dead” phenomena, rarely mentioned in the scientific literature, that can be a real part of a folk’s culture. In most countries, this concerns people in situations of social isolation.

The “living with the dead” phenomena is rarely mentioned in the scientific literature. It can be a real part of a culture. For instance, on the Indonesian island of Sulawesi, the Torajan people keep the bodies for weeks, months, or years in the family’s home, and they are fed and cared for as if they were alive. In the rest of the world, most often, it concerns people in situations of social isolation.

This case takes place in a small town near Rouen, France. An 82-year-old woman used to visit the city hall every Wednesday to pick up some packages. She did not go one time, which worried the employees of the town hall. They called firefighters, who, through the mid-open door of the old woman’s house, saw her lying on the floor, nearly unconscious. She immediately said that the dead body was upstairs. Firefighters finally entered the house by a small window on the first floor. Inspecting the house room by room, without any electricity, they found a body in a bathroom. It was a man’s body, mummified. The corpse was seated in the bathtub with his feet hanging over the edge. He was wearing wet pull-overs, and multiple cloths were spread over him. His hair was combed, and the corpse smelled like a mixture of ammonia and “eau de cologne.” After taking off the cloths, the investigators could see a yellow face, discolored. The woman, becoming aware, told the firefighters that her dead son was in the bathtub for six years, because nobody would help her with the burial. According to neighbors, this woman was widowed and was living with her mentally handicapped son.

A postmortem computed tomography and an autopsy were conducted on the male body. A lot of pupae were discovered, notably in the cranial cavity, but also some maggots and a spider under the left thigh. Some mold was found on and in the corps. The organs looked like a large mummified mass; only the kidneys were easy to identify, although dystrophic. The identity was suspected but not clearly determined. Therefore, the two femurs, the abdominal muscles, and both kidneys were taken for DNA analysis. On the postmortem computed tomography, the dental state could also help to determine the man’s identity. No trace of violence by any third party was found on the body, which was clearly in the bathtub for many years. The man probably died of natural causes and fell in the bath; his mother obviously could not move the body. Then, the elderly woman was hospitalized in a geriatric unit.

This incident is reminiscent of the Hitchcock story about Norman Bates and his dead mother, in reverse order. The discovery of mummified or skeletonized bodies in the domestic setting are not rare. In August 2016 in Italy, a mummified man’s body was found in a villa, two months after he died. The police were received by his wife, an elderly woman in evident confusion. Again, in southern Italy in the early 2010s, two women’s bodies were discovered in a country villa inhabited by three elderly, intensely religious sisters who developed a shared psychosis. The last surviving sister was in a very neglected state and was living with the mummified remains for more than a year. In these three cases, the main problem was the social isolation of elderly or mentally ill people, representative of an important societal ill. In this case, the neighbors thought the son was an autistic person, and nobody noticed his disappearance, neither administration, society, or neighbors.

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### Mummified, Living With the Dead, Isolation



## E42 A Forensic Approach to a Fatal Harbor Accident in Genoa, Italy, in 2013

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**Learning Overview:** The goal of this presentation is to show a morgue organization in the event of a single fatal accident with a large number of victims as well as stressing the concept of teamwork to guarantee an adequate service for the community, authorities, and victims' relatives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how important forensic investigation is in order to reach the correct postmortem diagnosis, even in the case of a traumatic accident, and to provide the best service to the judicial authorities concerning criminal and civil questions.

On May 7, 2013, at 11:30 p.m., a container ship crashed into a pilot control tower in the port of Genoa, causing it to collapse into the sea. Twelve persons were involved in the accident: nine died and three were wounded. The bodies were recovered in the sea, outside and inside the wreckage of the tower: the bodies of seven victims were found and taken to the town morgue of Genoa the day after the crash, another victim three days later, and the last one was found after ten days in the water. Victims' relatives arrived at the Departmental Section of Legal and Forensic Medicine, where the morgue is located in Genoa, the day after the accident.

Immediately, all the medical staff of the morgue worked in unison with harbor authorities, the Civil Protection Agency, psychologists, and the public prosecutor. After the authorization of the latter, all bodies underwent forensic examination to determine the cause of death: in accordance with Italian law, in five cases, a single gross examination sufficed as the traumatic injuries had forced brain tissue out from the skull, so revealing a clearly traumatic death. Also in the sixth case, the body arrived in the morgue dismembered after having been in the sea for ten days in addition to advanced decomposing phenomena and multiple lesions, both antemortem and postmortem; an external examination was performed.

In the last three cases, gross and internal examinations were performed; shaving lather froth and petechiae were present in all cases. Sectioning revealed marked lung edema and multi-organ congestion without fatal traumatic lesions. Consequently, the cause of death was attributed to drowning. In one case, tissue samples were collected in order to perform a microscopic investigation in the presence of a consultant appointed by the relatives of the victims. The consultant in question, during the Civil Court trial, aimed to demonstrate through the histological pattern the timing of death. To conclude, this presentation illustrates the vital role a correct forensic approach and teamwork involving different professional profiles has in providing sound evidence in both civil and criminal investigations and in offering adequate answers to relatives.

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### Forensic Approach, Traumatic Deaths, Teamwork

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## E43 Disclosing and Strengthening Forensic Science in Portugal: A Homicide Case

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**Learning Overview:** The goal of this presentation is to disclose, analyze, discuss, and by doing so, contribute to strengthening forensic science in Portugal.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an example of the wrong practice of forensic science concerning a case in Portugal. Attendees will understand how to correctly practice forensic science.

In a city near Lisbon, the capital of Portugal, a 51-year-old male triathlete disappeared on July 16, 2018, after his daily cycle training, according to his 43-year-old wife's statement to the authorities. The national media widely publicized his disappearance and, with the local people's help, his wife, family, and the authorities began a search. One month and nine days after the missing person report, the dead body of the triathlete was found skeletonized, with firearm aggression signs, 91 miles away from home (his cycle training starting point).

After the criminal investigation work of the judicial police, the authorities found a firearm at the house of the 43-year-old male lover of the triathlete's wife and, with the autopsy final results and conclusions, both were preemptively arrested on September 29, 2018. The court trial started on September 10, 2019, with a people's jury. On September 10, 2019, the first session of the court trial began with heavy media coverage and was passionately followed by the Portuguese population. After three months, the male lover of the triathlete's wife was released from jail.

Three months later, on March 3, 2020, the people's court decided to condemn the triathlete's wife to 25 years in prison (the maximum sentence in Portugal) for the murder of her husband and a not guilty decision for the lover was rendered, even though he owned the gun. On September 8, 2020, after the prosecutor's appeal, a superior court of law decided against the people's court decision and condemned the lover to the maximum sentence: 25 years for the murder of the triathlete, by using his gun. The higher court kept the sentence of the triathlete's wife by co-authorship. At present, both of the condemned are appealing to the higher court, and no one in the media or the population understands this decision. A lively national discussion about the justice system is currently underway.

There were multiple problems with this criminal investigation and court trial: the collection and value of evidence from the crime scene presented to the people's court, misinterpretation of the collected forensic evidence, the manner in which the forensic practitioners and the police agents testified in court, the lawyer's lack of knowledge and comprehension of the forensic evidence, and the Portuguese judge's and people's weak knowledge of forensics. To illustrate what was previously mentioned as autopsy procedure failures, a necropsy performed on August 8, 2018, and the exam conclusions misinterpreted by the people's court were examined. Despite the existence of a regulatory norm—NP-INMLCF-008, Recommendation n. R(99)3 of the European Council—that requires a radiological diagnosis (virtopsy) in firearm utilization cases, the medical examiner did not follow the correct procedures.

In addition, it was detected and mentioned in the medical examiner's report that he found a hyoid bone fracture. The medical examiner told the people's court that this injury was not relevant for determining the cause of death. The medical examiner did not mention whether the injury was caused antemortem, perimortem, or postmortem, nor was the court questioned. Result: doubts about the true cause of death.

Regarding the firearm and the use and misuse by the judge of a forensic exam: the forensic examination of the firearm and the projectile collected from the corpse were inconclusive. The court ruled that the weapon was responsible for firing the projectile, despite the forensic exam not so concluding. In the meantime, another event took place (February 14, 2020) that raised questions about the criminal investigation proficiency and the court's decision. Another firearm projectile was found in the house where the homicide presumably occurred (after the police analysis took place). During the trial, it was demonstrated that the police did not follow homicide case protocol. The aim of this real case presentation is to demonstrate that there are flaws in the application of justice in Portugal, flaws that can only be corrected if there is training and education in the field of forensic science. Both police actions and court decisions must follow scientific criteria and protocols because, if not, errors will prevail and the final decisions will be untruth, unaccountable, uncheckable, wrongful, and truly unfair!

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**Justice, Evidence, Untruth**

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## E44 Medicolegal Death Investigator Workplace Safety Hazards: A Scoping Review of the Literature

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**Learning Overview:** The goal of this presentation is to provide preliminary results of a scoping review addressing medicolegal death investigator workplace hazards.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a process for conducting a scoping review of a wide body of evidence to delineate eligible studies and identify knowledge gaps in the literature based upon a topic of interest.

Death investigators, because of dangers faced in on-scene investigations, have physical work requirements that may result in injury and illness that could lead to disability, absenteeism, or even death. Because death investigators face safety risks and occupational hazards similar to first responders (law enforcement officers, firefighters, emergency medical service, crime scene technicians), the research topic search was expanded to include these studies. The research team set a criterion for extraction to include research design and methodology, commonly occurring safety risks, reports dealing with physical and mental effects, and considered strategies for prevention to increase safety.

The research team used the framework proposed by Arksey and O'Malley and the Joanna Briggs Institute to guide the scoping review.<sup>1,2</sup> The five steps followed were to: (1) propose the question, (2) identify relevant studies, (3) select eligible studies, (4) document the evidence selected, and (5) summarize the results. The team proposed the question of: "What are the prevalent occupational safety risks and health-related conditions contributing to death investigator injury, disability, and death?" The pilot results from one database (Medline Complete [Ebsco]) retrieved or identified over 6,000 articles. Two independent researchers used Covidence software to identify relevant study citations by screening the title and abstract, excluding studies that dealt exclusively with occupational safety issues exclusive to law enforcement, firefighters, or emergency medical technicians. Covidence software was then used to manage the storage of the 43 full-text articles identified as eligible and to extract data from those articles.

The extracted data was stored on an abstraction form developed in Google® Forms. Of the 43 articles, seven met inclusion criteria. Of the seven, five (71.4%) utilized survey design, one (14.3%) was a systematic review, and one (14.3%) was a case study. The majority of the articles, six out of seven (85.7%), were specific to a wide range of employees within the setting of medical examiner or coroner offices. Of the seven articles, four (57.7%) addressed the emotional or mental health well-being of medical examiner and coroner office employees, which included death investigators. Three articles addressed occupational safety due to contagion from infectious disease, with three out of seven (42.9%) specifically addressed the forensic pathologist role, and one out of seven (14.3%) infectious disease safety among "death workers" in general. The findings of this pilot scoping review support the need to fill a gap in the literature addressing occupational safety of death investigators. These results serve as an initial step toward a more intensive investigation of death investigators' workplace safety.

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### Forensic Science, Medicolegal Death Investigator, Workplace Safety

## E45 Death on the Farm: Suicide by Tractor Runover

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**Learning Overview:** The goal of this presentation is to highlight the relevance of the work of the forensic pathologist and engineer in order to discover the real dynamics of the crime by joining their competencies in this rare case of suicide by tractor runover.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a rare case of suicide by tractor runover.

Several studies have found that in numerous countries across the world, agricultural workers, especially farmers, are at an elevated risk of suicide.<sup>1-3</sup> According to literature, firearms, hanging, precipitation, and poisoning are the most popular suicide methods among farmers, due to them being quite rapidly lethal and the means readily available. Agricultural machines, such as farm tractors, however, are seldom employed for suicidal purposes.<sup>4-6</sup> This study reports the nearly unique case of a man who committed suicide by letting himself get run over by his tractor.

A 48-year-old farmer was found dead in a field near his house. About 250 meters ahead of the body a crawler farm tractor was retrieved, with keys inserted in the panel and the gear engaged. The man had a long history of depression and was under pharmacological treatment. Given a recent worsening of his symptoms, his drug dosage had been increased by his psychiatrist. Moreover, he had repeatedly stated in front of his relatives that he often had suicidal thoughts. At the postmortem examination, observations included an otorrhagia from the left ear and multiple crushing wounds, such as bone fractures, contusions and abrasions, mostly located on the left side of the body; craniofacial and ribcage crushing; phalangeal fractures in the left hand; and abrasion and contusions on the left forearm. Toxicological exams revealed a therapeutic concentration of sertraline, as well as traces of oxazepam, in the blood. An engineering evaluation was also required, in order to assess whether it had been possible for the victim to start the tractor himself, then still have time to place himself on the trajectory of the moving machine.

Tractors are, undoubtedly, key equipment in farming because they can be used for transportation and transfer purposes, in addition to their agricultural use. On the other hand, farm tractors are the major cause of occupational fatalities in the agricultural industry.<sup>7,8</sup> Tractor-related deaths are typically the result of being run over or crushed by the tractor, becoming entangled in the moving parts of the machine, or accidents on roadways and tractor rollovers, which involve the tractor tipping sideways or backwards.<sup>9-11</sup> Agricultural machinery, however, is rarely used for suicidal purposes, because more quickly lethal and easily accessible methods are generally preferred. Tractor-related suicides are indeed so exceptional, that only one other case report was found, involving a farmer who beheaded himself using a tractor loader.<sup>12</sup> In this case, the combination of forensic and engineering investigation allowed not only the exclusion of any third-party implication, but also the assessment of the manner of death as a suicide: the man had repeatedly revealed to his relatives his suicidal intentions, even discussing the different methods he could use to enact them; the body showed no injuries, except for those consistent with a run-over by the machine; plus, the engineering evaluation stated that a tracked agricultural tractor, like the aforementioned one, is able to march “in abandonment” (driverless), further demonstrating the thesis of suicide.

As exemplified by this study, farm tractors, although only on rare occasions, can be used as suicidal means. In such cases, it is fundamental that forensic pathologists and engineers join their expertise in order to correctly assess the manner of death.

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### Tractor-Related Death, Tractor Runover, Farmer's Suicide

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## E46 A Remote-Controlled Flail Mower Work-Related Death: Who Handled the Controller?

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**Learning Overview:** After attending this presentation, attendees will focus on the difficulties correlated to the traumatic event reconstruction in cases of complex traumatic dynamics, especially if a composite machine or new technologies are involved.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of the collaboration between the forensic pathologist and the engineer in traumatic event reconstruction in cases of complex traumatic dynamics.

Since industrialization and the implementation of machinery's use in various kinds of work, forensic pathologists have had to face different types of work-related injuries. In the agricultural and forestry sector, tractors and self-propelled machinery are involved in many fatal accidents. In these cases, roll and runover accidents represent the most common cause of death.<sup>1-3</sup> More rarely, the literature describes fatal accidents with mowing machines (motor and brush mowers, flail mowers, shredders).<sup>4,5</sup> Regarding fatal flail mower injuries, only a case report in which an object mobilized by the mowers shoots the bystander operator have been published.<sup>6</sup> In this presentation, the case of a work-related death due to a fatal remote-controlled flail mower accident is presented.

A 57-year-old man was fatally run over by a remote-controlled flail mower while working with a colleague in a field. The forensic pathologist and the engineer arrived at the crime scene to understand the dynamics of the event. The visible lesions caused by the machine were: a huge laceration of the head with a rupture of the cranial theca and leakage of the brain; the amputation and near amputation of the right upper and lower limbs, respectively; a soft tissue laceration of the right scapular area; and several excoriations that mimicked the shape of the metallic part of the mower. A total body postmortem Computed Tomography (CT) was performed, revealing several ribs and other bone fractures, while internal organs were not injured, apart from the brain. The autopsy excluded any significant concomitant disease. The man's colleague informed the police that the victim was handling the mower controller.

In a case of work-related traumatic death, the judicial authority usually investigates to understand if there is a suspicion of manslaughter. For this reason, the exact reconstruction of the traumatic event has an essential role. The forensic pathologist, in collaboration with the engineer, has to piece together all the clues collected during both the crime scene examination and the autopsy. In this case, the main issue was to understand whether the accident was caused by a machinery malfunction or by human inattention. Then, if human inattention is proved, it should be investigated who was handling the machine controller. The engineering analysis excluded any machine malfunction and the controller did not present any damages or bloodstains. In accordance with the peculiar lesion pattern, the only reasonable hypothesis was that the man was run over while standing and the man's colleague was handling the controller, involuntarily causing the fatal accident.

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### Work-Related Death, Flail Mower Injuries, Unusual Lesion Pattern

## E47 A Silent Suicide

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**Learning Overview:** The goal of this presentation is to show an unusual case of suicide by crossbow bolt, in which radiological tools, alongside a complete autopsy, proved useful in reconstructing the intracorporeal trail of the arrow.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on the radiological strategic role for the reconstruction of the injury pattern in order to plan an approach to the autopsy.

Despite being common in the past, now lethal crossbow injuries are a rarity in the forensic field and refer mainly to accidents; homicidal events are less frequently reported, whereas suicides are only seldom encountered.<sup>1-8</sup> The head and chest are the areas predominantly involved; although cases regarding the perforation of the neck or abdomen, or combined injuries, are also described.<sup>9-13</sup> This case is a full-thickness chest injury caused by a bolt shot from a crossbow with suicidal intent.

A 48-year-old man was found dead in the fields surrounding his family house, with a three-bladed crossbow bolt protruding from his back. Near the corpse, an unloaded Skorpion Guillotine-X compound crossbow was retrieved. His relatives declared they had not heard the crossbow shot, and that they started looking for him only hours after he left the house, but when he was found, it was too late. During house inspection, the local police force discovered other crossbow bolts and a handwritten farewell letter. The postmortem Computed Tomography (CT) scan, along with a complete autopsy, allowed reconstruction of the intracorporeal path of the dart. The bolt entered the body, fracturing the xiphoid process; then, it proceeded on the sagittal plane, perforating the pericardial sac and the right ventricle, with subsequent hemopericardium and right hemothorax perforation. Ultimately, after going through the esophagus and the aorta, it caused the myelic fracture of T11.

This case is interesting from several points of view. First, the suicidal tool of choice, a crossbow: today, crossbows are mainly employed for sport or hunting because they are accurate and easy to use even without practice; their penetration power, due to the kinetic energy and the relatively high speed of the flying bolt, often exceeds that of firearms. Ballistic tests have demonstrated that sharp bolts can often puncture up to 80–90cm of living matter, and even penetrate bone (in this case, the arrow broke through a vertebra).<sup>14</sup> However, in many countries, including Italy, crossbows are considered by the law as “sports equipment,” meaning that, unlike firearms, anyone of legal age (18 years of age in Italy) can buy and own one without needing a specific license. In the literature, the chest is the second preferred target in both suicidal and homicidal shots, after the head; in our case, however, it is remarkable that the bolt pierced the full thickness of the chest. Unlike gunshot wounds, the characteristics of such lesions depend on the type of arrow, in particular the tips used, rather than on the type of weapon; and entrance and exit wounds could be virtually indistinguishable from distant gunshot wounds, if the arrow has been removed from the victim.<sup>15</sup> According to current literature, a three-blade tip produces stab and puncture wounds; thus, a three-pointed star-shape lesion, with clear-cut and blood-infiltrated edges, is generally found, as in this case, in which both gross examination and histology revealed perilesional hemorrhage (without relevant inflammatory infiltrate) where the arrow had passed through.

From a forensic point of view, crossbow-related deaths, especially suicides, may represent a rarity and a challenge. In such cases, the CT scan has proven to be useful because it allows easy reconstruction of the intracorporeal path of the arrow while it still is *in situ*, avoiding the risk of altering it; this risk, on the other hand, is tangible during autopsy, because the arrow must be removed or cut. Cases like the one here described highlight concerns regarding the accessibility of such weapons, which are accurate and easy to use even without practice, and, thus, should perhaps not be considered only as sports equipment.

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### **Crossbow Injury, Suicide, Forensic Radiology**

## E48 An Unusual Work-Related Death at the Construction Site: The Half-Twisted Man

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**Learning Overview:** The goal of this presentation is to present an unusual injury pattern caused by a spiral screw pre-feeder for fresh concrete. The lower half of the body appeared to be severely injured and peculiarly twisted on itself; the upper half of the body, however, seemed to be uninjured. A similar lesion pattern has never before been described.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an unusual pattern of injury in a work-related death.

Currently, the work-related fatal accident rate is slowly decreasing, but it still represents an important social concern.<sup>1,2</sup> According to the National Institute for Insurance against Accidents at Work (INAIL) of Italy, 1,089 Italian workers died from work-related injuries in 2019.<sup>3</sup> Construction industry workers seem to be at higher risk of death than other workers.<sup>4</sup> Often, the worker is killed when trapped in or between machines, equipment, or tools.<sup>5</sup> Unsafe behavior is one of the leading causes of work-related accidents in this field, and it has been suggested that a failure in the coordination between work operations may also have a role in increasing the occupational risk.<sup>6,7</sup> Herein, a case of an unusual work-related death in the construction sector is described.

A 48-year-old man was found entrapped in a spiral screw pre-feeder for fresh concrete by his colleagues during the daily work shift. The man was working with two of his colleagues when the fatality occurred. One of them started the machine for the production of fresh concrete, a twin-screw pre-feeder with two mixing axes, which was equipped with synchronized, counter-rotating spiral arms. The coworker, alarmed by terrible screams from the concrete mixer, stopped the machine immediately and found the man trapped between the two helical arms of the machine. The man remained alive for at least ten minutes after the emergency service's arrival but died during the rescue attempt. The judicial authority initiated an investigation and ordered the forensic pathologist to perform the autopsy. In such cases, the main issue was to describe the injuries found on the victim's body in order to reconstruct the traumatic event.

The machine involved in this case has two synchronized, counter-rotating, mixing spiral arms that produce different types of mechanical forces such as compression, traction, torsion, and shear stresses. This transfer of kinetic energy generates consequent tissue deformations and injuries with a peculiar pattern. In this case, extremely severe injuries were observed to the lower limbs, pelvis, and abdomen with minimal or no lesions on the upper side of the body.

The lower extremities seemed mutilated, skinned, and constricted to the point that a long operation was required to reposition the stumps. The right limb appeared to be completely amputated at the level of the proximal third of the thigh and ankle, with multiple fractures of the femur and tibia. The muscle layer of the right calf was completely exposed due to the complete skinning of the leg, and the skin seemed to be stretched upward and twisted around the longitudinal axis. The skin of the homolateral foot, however, seemed to be pressed down. The left limb, although not amputated, was also seriously injured, with several bone fractures and the absence of skin exposing the muscles. At the level of the femoral quadriceps, there were parallel oblique incisions. There were also comminuted fractures of the hip bone with complete exposure of the underlying muscles. Finally, there was a large abdominal breach. The edge of this abdominal lesion appeared excoriated and even here, parallel oblique incisions could be identified. The abdominal breach had caused several organs to leak out, including the small intestine and the omentum.

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### Work-Related Death, Torsion and Compression, Injury Pattern



## E49 How Many Times Does Forensic Pathology Have the Chance to Save Lives? A Case Report

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**Learning Overview:** The goal of this presentation is to show how autopsy and a complete genetic study play substantial roles not only in identifying the cause of death and serving justice, but also as a means of secondary prevention.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the importance of a familiar genetic investigation in cases of apparently unexplained Venous Thromboembolism (VTE) leading to sudden death. Genetic investigations can identify thrombophilic genetic mutations in patients with no other risk factors. This allows physicians to make a tailored plan of secondary prevention for relatives.

Secondary prevention aims to reduce the impact of a disease that has already occurred by detecting and treating pathology as soon as possible to halt or slow its progress and to prevent recurrence or long-term complications. Forensic pathology can rarely boast of playing a direct role in this context and, as far as sudden death is concerned, this is even more true.

VTE, consisting of Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE), plays an important role in sudden death, being the cause in approximately 15% of cases, exceeding the mortality rate for acute myocardial infarction, with a big socio-economic impact affecting millions of individuals worldwide. Half of the cases are idiopathic and occur without previous trauma, surgery, immobilization, or cancer. Several gene polymorphisms associate independently with an increased risk of VTE, among which the most common is Factor V Leiden (G1691A). Diagnosis of VTE can be a great challenge as its signs and symptoms are non-specific, constituting a large part of the misdiagnosis at autopsy.

The presented case concerns a 31-year-old Chilean man adopted with his two brothers by Italian parents. He was admitted to the local emergency department complaining of cough and dyspnea. His anamnesis was mute and his own family history was not achievable. The main vital parameters were: oxygen saturation 97%; heart rate 108/min, and blood pressure 110/80mmHg. Physical examination revealed reduced breath sounds, diffuse rhonchi, and wheezing. Echocardiogram, Electrocardiogram (ECG), and an arterial blood gas test were within normal limits. A thickening of right lung interstitial was observed at X-ray. Clinical chemistry reported an increase in D-Dimer (4,252ng/ml) and lactate dehydrogenase (569UI/L). A chest CT was advised but not performed because it was refused by the patient, who asked to be discharged. Two days later, he was found dead in his apartment.

The external examination only revealed a contusion in the parietal region of the head. During the autopsy, an embolus was identified in the pulmonary trunk, extending in its two branches, associated to pulmonary edema and infarct. At the examination of the lower limb vessels, a right popliteal vein thrombus was found. The toxicological analysis on blood and urine were negative. Because of the young age of the patient, his low VTE risk according to Wells criteria, and D-dimer increase, a genetic investigation was conducted on a spleen specimen collected during the autopsy. A Factor II gene mutation (IVS13 ds + 88 A > G) in heterozygosis was discovered. In the literature, this allele results in rising plasma prothrombin levels, constituting a pathological substrate on which a trigger event determines a higher risk of VTE.

In order to provide an early diagnosis of the family members, his two brothers and two sons underwent a specific genetic investigation. Among these, one single brother was proven to possess the same mutation as the dead man. This genetic finding allowed him to be informed about his thrombophilic haplotype, about interaction between life style and genetic factors, and about all risks of VTE, giving him the possibility of making informed choices about the most effective means to prevent disease and its complication, thus *Pursuing Justice* for his relatives *Through Truth in Evidence*.

### Venous Thromboembolism, Sudden and Unexpected Death, Secondary Prevention

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## **E50 How to Get Away With Murder: The Importance of a Correct Reconstruction of a Crime Scene Investigation (CSI)**

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**Learning Overview:** The goal of this presentation is to explain the importance of using a scientific method to solve investigative questions in unclear crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the accurate collection of scientific evidence represents the keystone for discovering a crime.

In many countries, a local physician is the first figure that acts at the discovery site of a corpse. In these cases, the external examination is the principal tool adopted to look for the cause of death. This approach can be imprecise, as only the autopsy can reveal the exact mechanism of death. Furthermore, a doctor who is not a forensic pathology expert tends not to pay attention to the state of the discovery site of the body. This can lead to incorrect conclusions on the modalities of death.

The presented case concerns a 50-year-old man found dead on his restaurant floor, near the rolling gate. Police site inspection revealed a ladder leaning against the wall under the rolling gate box. An electric cable hung from this ladder. The emergency responder said the cause of death was due to electrocution associated with fall trauma. Initially, the death was classified as an accidental event. Nevertheless, the prosecutor required the intervention of a team of forensic pathologists to perform the site inspection and the external exam of the corpse.

When the forensic pathologists arrived on the scene, they noticed that the cable was intact, with no knots, and was not connected to any electrical source. External examination of the corpse revealed the absence of electric marks on the skin and the presence of a ligature mark on the neck. According to the forensic pathologists, the ligature mark characteristics did not correspond to the electric cable found on the scene. No other traumatic lesions were found.

After the preliminary cadaveric inspection, the prosecutor opened a judicial file and required an autopsy. The autopsy showed hemorrhagic infarction areas of the muscles of the neck and the right parotid gland and a fracture of the right posterior horn of the hyoid bone. Histological examinations confirmed erythrocytes in the neck muscle samples; no wavy appearance or fragmentation of the myocardial fibers was found. Immunohistochemical examination performed on the skin sample of the ligature mark showed positivity to CD15, tryptase, and IL-15, demonstrating the vitality of the lesion. The positivity of human anti-hypoxia-induced factor 1- $\alpha$  (HIF1- $\alpha$ ) on endothelial cells of the lung vessels confirmed the asphyctic death. Toxicological exams revealed positivity to high levels of alcohol and cannabinoids in the blood. In conclusion, the evidence derived from crime scene data (external examination, autopsy, histological, immunohistochemical and toxicological exams) contributed to excluding an accidental event and corroborated the hypothesis of homicide. The death was attributed to strangulation. Further inspection of the places surrounding the restaurant did not allow the scientific police to find other ligatures compatible with the mark on the neck. Investigations are still ongoing.

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### **Strangulation, Crime Scene Investigation, Ligature Mark**

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## E51 Murder? No! It Is Just Drug Abuse: A Weird Crime Scene

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**Learning Overview:** The goal of this presentation is to underline the relevance of integration of evidence collected by forensic pathologists during crime scene investigations, autopsies, and toxicological examinations. This evidence is fundamental to establish the cause of death and to settle the investigations, orienting them on the right path. Such orientation is particularly relevant in cases in which circumstances are unclear and the determination of the forensic pathologist can help address the investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that the autopsy and the toxicological examination are crucial to guide police investigations, particularly in cases where the crime scene investigation led to a different dynamic of events. They are all fundamental tools in the research and the collection of evidence and samples.

The presented case dealt with a 37-year-old man found dead next to a peripheral road by a passerby. The corpse was in a prone position, wearing only underwear and a sock. The prosecutor called the coroner to carry out the crime scene investigation and the corpse external examination. The external examination showed no injury of the back, multiple bruises and excoriations of the anterior part of the trunk, a lacerated wound of the chin, extensive excoriated complexes on the lower limbs (especially on the knees), and a linear wound on the scrotum. The thanatological parameters, collected on the spot, indicated that the death had occurred a few hours before the corpse examination. The lesions appeared to be plural, multidistrict, and polymorphous. These characteristics are typical of the application of contusive forces, being indicative of a trauma. The examination of the clothes revealed a piece of fabric missing from the crotch of the underpants. The missing piece of cloth coincided with the scrotum wound. At the end of the corpse external examination, the coroner took a sample of blood and urine.

The evidence gathered during the crime scene investigation and the external examination suggested a traumatic death. Police investigations also focused on a homicidal dynamic. During the investigation, the clothes of the dead man were found near the discovery site; a small fragment of fabric was found entangled in a net. This piece was compared to the fabric of the underwear of the deceased, showing that they came from the same garment. The interrogation of the wife revealed that her husband was a cocaine and marijuana abuser, and the man left home suddenly during the night.

The autopsy was performed, with unexpected results. Only small hemorrhages to the pectoral muscles, abundant stasis, and visceral congestion were found. No other traumatic injuries to internal organs were found. The toxicological analysis was necessary. Samples of biological fluids (blood, urine, and bile) were again collected, such as small portions of parenchymal organs (brain, liver and kidney). The first toxicological screening showed positivity to cocaine, cannabinoids and alcohol. Histological examination revealed widespread stasis and congestion of all tissues, with no microscopic hemorrhages. The autopsy and toxicological examination showed a death related to drug abuse, thus excluding the hypothesis of murder. In conclusion, the autopsic and toxicological examinations were decisive to guide the investigation and to reconstruct the dynamic of the event. It is evident the importance of integrating the findings from all the forensic team investigations in order to answer to judge questions.

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### Autopsy, Drug Abuse-Related Death, Toxicological Exam

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## E52 Men on Fire! Two Murders Solved by a Multidisciplinary Approach

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**Learning Overview:** The goal of this presentation is to highlight the importance of a multidisciplinary approach in complex cases. The scientific method represents an essential tool in crimes in which the murderer tries to conceal the homicide in order to help the court reconstruct the dynamics of the events.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that in criminal cases, the use of a multidisciplinary approach is essential. This approach involves different specialists, depending on the cases, to guarantee a complete and correct scientific evaluation to support the investigation.

The concealment of the body following a homicide is a frequent occurrence in forensic pathology. Killers can hide the body of the victim to destroy crucial evidence and divert the investigation. There are various ways of concealment from simple abandonment in an isolated place to dismemberment. In many cases, it can be difficult to distinguish between antemortem and postmortem injuries and to find the cause of death. Two cases of murder concealed by carbonization analyzed by the Forensic Pathology Department of Foggia are presented.

The first case concerns an unknown corpse, found charred in an isolated countryside, on the left side of a burned car. The coroner, called by the prosecutor, inspected the body: the bones of the skull roof were destroyed, while part of the skull base and the mandibular bone and soft tissue were recognizable. The thoracic and abdominal walls were absent, such as the heart, lungs, and spleen. Due to the position of the corpse (it laid on the ground between the open car doors), the police assumed an accidental death or a suicide. According to the accidental hypothesis, the person could not escape from the sudden fire of the vehicle. On the other hand, the person could have set fire to the car and taken drugs to lose consciousness.

The forensic pathologist required a total body Computed Tomography (CT) scan. The CT scans showed metal objects in the mandible and in the thighs, maybe belonging to the car. So, an autopsy was performed. The pathologist found a rounded metal body in the soft tissue of the mandible. Carbon residues were not found on tracheal mucosa. The forensic pathologist understood that the unknown person had already died before the car fire. The forensic pathologist delivered the metal fragment to the investigative scientific core. The laboratory technicians discovered that the object was the lead core of a 7.65mm Browning® bullet. By combining all the evidence, the police had a new hypothesis: someone killed, then burned the man to conceal the body and divert the investigation.

The second case concerns the body of a man found inside a warehouse, behind a burnt car. The prosecutor requested the intervention of the forensic pathologist. The corpse laid prone on the flame-burned floor. Only the scalp, the skin of part of the face, and the back were charred. The external examination revealed the presence of a hole in the occipital region, with scarcely evident features due to the carbonization of the scalp, the absence of the right upper central incisor tooth, and hemorrhagic infarction of soft tissues of the right part of the oral cavity. A total body radiological examination performed before the autopsy showed the presence of two metal bodies, placed in the frontal region of the brain and anterior to the odontoid process of axis vertebrae, respectively. The autopsy determined that one of the two bullets had entered the right occipital lobe, right cerebellar hemisphere, brain stem, optic chiasm, and left frontal lobe of the brain. The second bullet was found in the prevertebral region, anterior to the axis. Blood was found in the lumen of the trachea and the main bronchi. Histological stains performed with hematoxylin-eosin showed the presence of subarachnoid hemorrhage in the samples taken from cerebral holes, erythrocytes between the muscle fibers in the sample taken from the oral cavity, and the absence of carbon particles in the lumen of the trachea. The sodium rhodizonate test performed on the skin sample taken from the hole in the occipital region was negative. There were no traces of carbon monoxide in the blood. By using a multidisciplinary approach, the forensic pathologist determined that the cause of death was due to cranioencephalic trauma caused by two gunshots and confirmed that combustion occurred postmortem. Subsequent police investigations revealed that the murderer set fire to the warehouse to conceal the body. Unfortunately, a passerby called the firemen who extinguished the fire and found the body.

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### Murder, Carbonization, Corpse Concealment

## E53 An Examination of the Self-Efficacy of High School Students in the Prevention of Drug Addiction

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**Learning Overview:** After attending this presentation, attendees will understand the importance of adding a “self-efficacy development” step to addiction prevention activities along with diagnosis and treatment studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a new approach to the prevention of substance abuse in high school students.

Body and thought structures show rapid development under the influence of hormones. Adolescents may have difficulty keeping up with these developments and changes. In addition, at a time when they feel complex themselves, being understood by family and the environment is one of the most important factors for them to easily overcome the difficulties of adolescence.<sup>1</sup> Because adolescence is a period of young people’s transition to independence and proving themselves, the risks of starting substance use and involvement in crime are most often seen during this period. From this point of view, individuals aged 15–25 years of age constitute the risk group.<sup>2</sup> The most common causes of substance use are a sense of curiosity, the impact of the social environment, and the state of the family, which can be shown as an effort to create an independent identity.<sup>3</sup> When the processes of fighting substance abuse are examined “treatment,” “protection/prevention,” and “awareness building” steps are encountered. Nevertheless, not including the notion of “self-efficacy” in this process weakens the fight against substance abuse.

When the current literature about substance abuse is examined, it seems that most of the studies are conducted on the treatment of substance abuse or the study of people who are addicted to drugs already. The aim of this study is to demonstrate the importance of self-efficacy against substance and addiction in individuals who, unlike other studies, have not yet become addicted or have yet been introduced to substances and to bring a new perspective in the fight against substance abuse. This study was applied to students (grades 9, 10, 11, and 12) in secondary education institutions located in the Esenyurt district, Istanbul province. A total of 421 students participated in the study. But 84 of them were not included in the study because their scales were incomplete and inconsistent. Limited “Socio-Demographic Data” forms and “Self-Efficacy Scale in Substance Abuse Protection” were used to obtain data from the students involved in the study. The findings were reached through the Statistical Package for the Social Sciences (SPSS) program. Distributions of variables were evaluated using the Kolmogorov Smirnov test. Cross-group comparisons of variables were performed using the Mann Whitney U and Kruskal Wallis tests. Determining the linear relationship between variables was performed using the Spearman correlation test. A statistical significance value of  $p < 0.05$  was accepted.

According to the results obtained from the data, although there are no statistically significant differences between participants when evaluated according to gender and the grade of the class in progress, schoolgirls’ and 12<sup>th</sup>-grade classroom students’ self-efficacy levels were found to be higher than others. At the same time, there were no statistically significant differences in the state of parent cohabitation and the state of psychiatric illness in the family, but it was concluded that the self-efficacy levels of participants whose family lived together and whose family did not have psychiatric illness were higher than others. In the responses to the control question, which are crucial for measuring the self-efficacy levels of the scale, it is also seen that the self-efficacy levels of the participants are weak.

In order to strengthen the findings of the study conducted, it was compared with the findings of previous studies, and it was observed that the findings were equivalent to the studies in the literature. As a result of the evaluations obtained, it can be seen that the adolescent community formed by the participants does not have the level of self-efficacy in terms of protection from substance abuse. In this sense, “self-efficacy development”—which should be organized by educational institutions, non-governmental organizations, security units, visual and written media, ministries that work with young people, and, especially, in the family—need to be added to prevention activities along with diagnosis and treatment studies.

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### Crime Prevention, Addiction, Self-Efficacy

## E54 Ethical Committee: A Southern Italian Experience

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**Learning Overview:** The goal of this presentation is to illustrate the results obtained by the Regional Unique Committee (CEUR) of Basilicata, a region of southern Italy, published in 2018 in response to the transposition of the European Directive on the evaluation of studies in the health sector.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the European Parliament directive for the protection of public health and the progression of medical and biological sciences have been implemented in Italy, particularly in Basilicata.

In Directive 2001/20/CE of the European Parliament, the Ethics Committee is defined as an independent body, made up of health and non-health personnel, responsible for guaranteeing the protection of the rights, safety, and well-being of trial subjects and providing public guarantees of this protection, issuing, for example, opinions on the testing protocols, the suitability of the investigator(s), the structures, and on the methods and documents used to inform the trial subjects before obtaining informed consent. In Italy, the establishment of Ethics Committees is provided by the law in public health facilities, private hospitals, and care institutions. The purpose is to guarantee the feasibility of a research project in terms of ethical and scientific correctness of the experimentation, the protection of the rights of those taking part in the clinical study, and the adequacy of the relationships between the center where the research is conducted and the sponsor of the study. The Ethics Committee may express a favorable or unfavorable opinion. The deadline for the expression of this opinion is 30 days from the presentation of the request of the research promoter. The Ethics Committee communicates its opinion to the Ministry of Health in this time frame. After having obtained a favorable opinion from the Ethics Committee (and if the competent authorities have not communicated motivated objections), the clinical trial can begin.

In Basilicata, a little region in South Italy, the CEUR was established by the Regional Law of 4 August 2011, n. 17 to make a single regional in-depth study on the bioethical aspects and all their ethical and legal implications connected with the health care and biomedical research and its environmental impact.

The provision of the Regional Council Resolution n. 930 of 10 July 2012 established that the function of the CEUR was to provide consultative activities in matters within its competence in favor of health care practice and biomedical research; to propose legislative and administrative solutions in the same subjects; to promote a bioethical culture in the regional territory and the development of bioethical sensitivity in health workers and the population; to increase the quality and safety levels of the health service; to guarantee the development of an organic and constant function of study and research, training, and education; scientific reference and advice in the field of bioethics; to take care of the relationships between local and national institutions interested in bioethical issues; and to provide advice for the Regional and National Council, if required.

In Basilicata, four hospitals ask CEUR for advice: a Scientific Research and Health Care Institute (called C.R.O.B.), a regional hospital (called “San Carlo” hospital), and two local health authorities (called ASP and ASM). In 2018 the approved studies were 92, among which 39 were proposed by the “San Carlo” Hospital, 35 by the C.R.O.B, 17 by the ASM, and 1 by the ASP. These 92 studies are divided into drug trials: 27 out of 92, of these, 10 were financed and 17 were non-profit, observational studies; 49 out of 92, of these five were financed and 44 were non-profit; compassionate use of drugs, 16 out of 92, not surprisingly all of them were non-profit.

The data concerning each hospital are summarized: “San Carlo” Hospital proposed 12 experiments with drugs (of which 4 were financed and 8 were non-profit); 24 observational studies (of which 3 were financed and 21 were non-profit), three therapeutic use of drugs; C.R.O.B. proposed 13 experiments with drugs, of which six were financed and seven were non-profit; 12 observational studies, of which 1 was financed and 11 were non-profit; 10 therapeutic use of drugs; ASM proposed 2 non-profit experiments with drugs, 12 non-profit observational studies, 3 therapeutic use of drugs; ASP proposed 1 financed observational study.

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### Ethics Committee, European Parliament Directive, Basilicata Data

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## E55 Closed-Circuit Television (CCTV) Detection of National Security Threats Through On-Screen Identification of Text

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**Learning Overview:** After attending this presentation, attendees will understand the current problem within digital forensic data analysis in relation to the retrieval of text messages due to advancements in encryption in order to protect the privacy of the users. To combat this problem, current research has proposed a way to utilize the CCTV and devise a framework to guide which type of CCTV to use and how to configure the CCTV to support the task of screen monitoring on a personal mobile device (smart phone).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a novel approach to using CCTV aid with national security, not only against terrorists but also against any other illegal activities that would threaten the infrastructure of the country. Therefore, the ability of reading text on the phone screen could be exceptionally useful for anti-terrorism task forces.

As mobile phones are currently playing an important role in crime, from being used as a device for plotting bad deeds to being used to combat it, in the current academic literature, there is little coverage on the misuse of mobile phone for such purposes. As criminals change their communication and planning tactics, law enforcement has to think of a way to come up with preventive measures accordingly. Having said that, it is not the case that the current technology is incapable of achieving such a task, but it is more the case of not using the current technology to its maximum potential. This research aims to examine the usability of CCTV in a high traffic area (such as the airport) to test its impact on monitoring the content on a personal device.

The experiments have not proceeded to completion; however, the current results show promising future research for CCTV in this direction. With the usage of a 2.8mm 6MP Hikvision® EXIR Turret Network camera, text messages on the Messenger application on a Samsung® A70 phone can be read at brightness levels between 10%–70% from a distance of 1m. Best results can be obtained at 20%–30% brightness, which is a common phone setting while being indoor (in auto-adjust brightness mode). The best font size that can be picked up by the camera is between level 6 and level 8 (5mm–6mm text). Screen zoom is a function on Samsung® phones that is used to adjust the size of the content on the phone, such as text and User Interface (UI) elements. Screen zoom was used in this experiment as an attempt to further enhance or minimize the text. However, experiment results showed that it had no significant effect on the ability of the camera to pick up text on the phone screen. There were also no significant differences between capitalized text vs. normal text or bold text vs. normal text. Finally, there was no noticeable difference between dark mode and light mode in text detection between 10%–70% brightness.

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### CCTV, Phone Screen, Text Monitor

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**E56 Two Cases of Sharp Force Trauma: Homicide or Suicide? Solving an Enigma With Forensic Methodology**

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**Learning Overview:** The goal of this presentation is to describe two unusual cases of death due to sharp force trauma. The scientific method represents an essential tool in order to solve an enigma between homicide or suicide. After attending this presentation, attendees will appreciate the typical pattern of sharp injuries, identifying, in observed sharp force fatalities, reliable parameters that can differentiate a homicidal and suicidal manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of forensic methodology in solving diagnostic dilemmas between homicidal or suicidal fatalities due to sharp injuries. Crime scene investigation, a detailed postmortem examination, and a complete histopathological study are mandatory to solve the enigma.

Sharp force trauma is a popular cause of homicide and suicide in many countries. Characterization of the injuries between the two is crucial for a differential diagnosis. Suicidal wounds are typically multiple, favor certain sites of election—such as throat, wrist, and front chest—and often characterized by a number of preliminary trial cuts, called “tentative incisions.” Nevertheless, the interpretation of sharp force fatality dynamics may be difficult in some cases, but a contribution to analysis of the phenomenon may be provided by case studies. Therefore, this presentation discusses two cases of suicide concealed by sharp force trauma: the first is characterized by 44 sharp wounds and the second by only one sharp wound. The wide variety between these two cases makes this presentation an opportunity.

The first case concerns a 44-year-old man who was found dead in his house near Rome. Police officers and forensic pathologists were immediately alerted to investigate the crime scene. The man was found nearly naked, lifeless, lying on the floor in the bathroom. A big pool of fresh blood was detected on the floor, near the cadaver. Two big knives (length: 44cm and 20cm, respectively) were found on the sink. In the past, the man had been charged with child abuse and was under house arrest. A complete postmortem investigation was performed the day after death. Twenty-two sharp wounds were described on his chest and abdomen, and 20 more sharp wounds were described on his arms (“tentative incisions”). At gross internal examination, hemorrhagic infiltration in the subcutaneous tissues of the chest and sharp wounds of the heart and both lungs were observed. None of the abdominal organs were involved. Histological stains performed with hematoxylin-eosin showed erythrocytes between the muscle fibers in the sample taken by the heart as well in the pulmonary alveolus in the sample taken by the lungs. These demonstrate the presence of tentative incisions, which support a suicide hypothesis by knife wounds.

The second case concerns a 73-year-old man who self-stabbed himself in his house in Rome. His wife was present and tried to aid him by removing the knife from his chest. The interrogation of the wife revealed that her husband was an alcoholic and had a history of depressive disorder. A wide, deep, incised stab wound with regular margins was observed in the anterior region of the chest at external examination. No other traumatic injuries were detected. A complete postmortem investigation was performed the day after death.

At gross internal examination, mild cerebral edema was recorded. Gross examination of the chest was performed according to conventional techniques and showed massive hemorrhagic infiltration in the subcutaneous tissues. A near-complete heart laceration was observed in correspondence of the left ventricle. Unexpectedly, lacerations of the heart were present on a big fibrous area. The lungs were not involved in the injuries. White foam was observed at the airways exploration. Both cerebral vasogenic and pulmonary edema was observed at histopathological examination. Huge amounts of erythrocytes were detected in the skin samples and heart samples. Foci of contraction band necrosis were also observed on the heart microscopic examination. Toxicological analysis was suggestive of mild alcohol intoxication. The cause of the death was massive hemorrhage. Both morphological and microscopical evidence from autopsies and information obtained by a detailed crime scene investigation led the investigators to conclude a successful and atypical sharp force suicide.

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**Suicide, Sharp Force Trauma, Self-Stubbing**



## E57 Men Who Hate Women: The New Italian Stalking Law and Femicide Cases Review in Foggia Territory

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**Learning Overview:** The goal of this presentation is to increase the understanding of the evolution of the Italian scenario of femicide after the enactment of a new Italian law on stalking.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting information about the introduction of the new Italian legislation and its effects on the reduction of cases of femicide, as reported by the Italian Ministry of the Interior. However, in Foggia, an increase of femicides was observed, resulting in discordance with the data of the Italian Ministry of the Interior.

In 2009, the crime of persecutory-stalking was introduced; these crimes include any violent acts or persecutory attitude that force the victim to change their way of life. This law was updated with law no. 69/2019 on the protection of victims of domestic and gender-based violence. This law introduces the so-called “Red Code,” which provides for the establishment of a privileged lane for investigations, with more severe penalties for crimes committed in the family or cohabitation, as well as for crimes of sexual violence and stalking.

Law no. 69/19 consists of 21 articles, including those of abuse of family members or cohabitants (Article. 572, Penal Code); sexual violence (Articles 609 *bis*, *ter*, and *octies*, Penal Code); sexual acts with minors (Article 609 quarter, Penal Code); fraud of minors (Article 609 *quinquies*, Penal Code); persecutory acts (Article 612 *bis*, Penal Code); revenge-porn (Article 612 *ter*, Penal Code); deformation of the appearance of a person through permanent injuries to the face (Articles 582, 583 *quinquies*, Penal Code). In terms of substantive criminal law, the Red Code provides for the exacerbation of penalties for those offenses listed above.

This new law has once again shed light on cases of femicide, defined as an extreme act of violence against a woman perpetrated by a man that often occurs within a relationship or cohabitation. About 150 cases of femicide were recorded every year in Italy (157 in 2012, 179 in 2013, 152 in 2014, 141 in 2015, and 145 in 2016), for a total of about 750 homicides in these five years. A survey of murder sentences of women issued between 2012 and 2016, highlights that out of 417 judgments examined, 355 are classifiable as femicide, which represents 85% of cases. The analysis of the sentences shows that in 87.9% of cases, the relationship between perpetrator and victim is one to one; 9.1% of cases highlighted a murderer with more victims (very often, they are minor children), while 12.1% concern episodes with several murderers versus one or more victims. Men are the perpetrators in 98% of cases. In 55.8% of cases between the murderer; in 15.1%, they are friends; in only 2.2% of the cases are the murderer and victim colleagues or employers; while in 9.4% of cases, the victim and the killer do not know each other (cases of prostitutes or elderly ladies living alone). The weapon mainly used is the knife. In almost half of the cases examined, it was the perpetrator of the femicide himself who gave the alarm and notified the police.

In this scenario, the Department of Forensic Pathology of the University of Foggia conducted a review of all cases of femicide in the territory of Foggia. The autopsies performed, from 2001 to 2020, were analyzed with the goal of selecting which and how many of these were murders that had female victims, evaluating the mechanism of death, and the history of the sentimental relationship between the victim and the offender. This evaluation indicated that 27 femicides were committed by the husband (8), intimate partner (6), ex-husband (1), son (2), father (2), family member (3), a friend (1), and unknown people (3). Of the 27 femicides, 3 presented blunt injuries, 5 stab wounds (1 with decapitation), 12 gunshot wounds, 5 asphyxial mechanism (one with drowning, two by suffocation, and 2 by strangulation), and 1 due to a fall during beatings.

### Femicide, Italian Data, Stalking Law

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## E58 Football Hooliganism: A Case of Homicide by Being Struck by a Car

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**Learning Overview:** The goal of this presentation is to underline the importance of the forensic pathologist's role during the reconstruction of a crime scene. This is more frequently required in the case of violent deaths, especially suspected murders; he/she needs to apply a critical scientific mind to identify staged crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing how the crime scene examination carried out by the forensic pathologist was the most important event in directing the investigation. A second look at non-organic elements from the same scene revealed what had really taken place.

Football (Soccer) hooliganism is a significant social burden in Italy, often characterized by episodes of vandalism and public disorder, not infrequently ending in death. The presented case report concerns a murder of a football supporter by a group of the opposing team during an ambush. In particular, the reconstruction of the homicide's dynamic by the police found that the man was run over while trying to attack the driver after starting a fight. The crime scene showed the presence of two cars placed one in front of the other, both with broken bodywork and broken glass, one of them with a specific sign of pedestrian loading on the anterior part and on the windshield with blood traces. The corpse was found about 20m from this car. Also, several wands were found on the road all around.

External examination of the corpse revealed a typical pedestrian distribution of lesions (bruises and wounds) of the lower limbs; moreover, multiple blunt injuries were found on the whole body, compatible with the use of the wand. The autopsy confirmed the lesions mentioned above, a relevant head injury with skull fractures and brain hemorrhage as the likely cause of death, and a chest trauma with multiple rib fractures. A large number of injuries in different parts of the body (affecting the head, thorax, abdomen, arms, and brain samples showed that all injuries were vital. These characteristics are typical of the application of "contusive" forces, being indicative of great trauma suggestive of road traffic accidents and were compatible both with the projection onto and with the impact of the body against external car parts and the asphalt, indicative of a car hitting a pedestrian, but showing also multiple blunt injuries of the body by a different tool like a wand.

The goal of this case report is to underline the importance of the forensic pathologist's role during the reconstruction of a crime scene. This is more frequently required in the case of violent deaths, especially suspected murders; he/she needs to apply a critical scientific mind to identify staged crime scenes. In this case, the evidence provided by the autopsy and witnesses questioned by the police officers were not decisive in resolving the case. Indeed, the crime scene examination carried out by the forensic pathologist was the most important in directing the investigation, and a second look at non-organic elements from the same scene revealed what had really taken place.

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### Struck by a Car, Crime Scene Examination, Ambush

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## E59 A Complex Suicide Committed With a Knife and a Blunt Object

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**Learning Overview:** The goal of this presentation is to show how the presence of the forensic pathologist at the crime scene investigation can be essential in cases of complex suicides and assaults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the intervention of the forensic pathologist at the crime scene can be essential to the olive investigation. In fact, in this case, the stone would likely not have been noticed and would have been consequently lost. A person without expertise in wound recognition would attribute the wounds only to the knife. In addition, this presentation refers to a case of suicide committed by lapidation, which is a rare occurrence.

A 65-year-old man was found dead in his car. The man had been missing since the previous day, and the car was found in the countryside. The car's interior and the corpse were covered in blood. There were also blood traces on the external body of the car, on the trunk, and along the whole left side. Additionally, blood traces were on the ground along the left side of the car.

The dead man was lying across the driver's seat with his legs outside the car. Near the body, inside the car, there was a common kitchen knife (with a ceramic blade of approximately 10cm in length). The police and the prosecutor suspected a murder and called the forensic pathologist to the crime scene. The external examination of the corpse showed multiple wounds on the top of the head and cranial bones were partially exposed; on the left forearm, there was a penetrating wound. According to the forensic pathologist, the wounds on the head did not look like typical knife wounds. So, the forensic pathologist requested searching for other possible weapons at the crime scene. Near the body, in a pool of blood, there was a small stone. This stone was smooth, 200–300gr in weight, and covered in blood. The police took it for laboratory examination.

An autopsy was performed and showed a cranial fracture but with no type of cerebral hemorrhage. There were no signs of the knife on the cranial bones, and the scalp wounds looked like typical wounds caused by a blunt object. Only the wound of the left forearm was clearly compatible with the knife found at the crime scene. The cause of death was acute blood loss. Consequently, the forensic pathologist established that the knife was used only to wound the forearm; indeed, the wounds on the head were produced via several strikes of a blunt object compatible with the stone found in the car. Police investigations established that the man killed himself.

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### Complex Suicide, Lapidating, Crime Scene

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## E60 A Drowned Man in the Woods: A Case of Death by Drowning in Blood

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**Learning Overview:** The goal of this presentation is to show how wounds on the neck due to sharp weapons cause death in ways other than damage to large blood vessels or the trachea.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how autoptic examination is always necessary in cases of neck wounds. In fact, the vital structures, such as greater blood vessels, nerves, and airways in the human neck, are well protected by anatomical structure. Consequently, in many cases the wounds in the neck are not the real cause of death. In this case, if an autopsy had not been performed, the police would have excluded the hypothesis of suicide because a wound to the trachea or neck blood vessel by a huge knife would cause the death in a few minutes without medical intervention.

A woman called police and emergency medical services stating that her husband had stabbed his own neck with a long knife and then fled in his car. She also said that she did not know where he was going. Police started the search for the missing man. His car was found half an hour's drive from the city. The car had been parked along a countryside road and was locked. There was no trace of the man. In the woods nearby, the man was found dead, lying on the ground, with a long knife near by his body. There were several wounds on his neck and a red foam exiting from his mouth. The wife of the dead man also said that her husband had suffered from severe depression for many years. Regardless, the police and the prosecutor suspected murder, because they did not understand how a man could run and drive after a severe stab wound to the neck.

An autopsy was performed. On the neck were several wounds that looked like typical penetrating knife wounds. The forensic pathologist examined the knife found near the body: the blade was 20cm long and the point was bifurcated. The examination of the tissue of the neck showed, surprisingly, no lesions of great blood vessels, nerves, and upper airways. Instead, the blade had penetrated the soft tissue of the neck and mouth, going through the tongue. The examination of the upper airway confirmed that there were no sharp lesions, but the mouth and lumen of the trachea were full of foamy blood. Pulmonary airways were full of blood, too. The cause of the death was established as due to the sharp lesion in the mouth, not because of damage to vital structures in the neck, but by damage to the tongue causing a large release of blood. Consequently, the man died because of massive blood release in the airways and he drowned. The release of blood started when the man extracted the knife buried up to the handle in his neck.

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### Suicide, Neck Stab Wound, Drowning

## E61 The Use of Video Recording Systems in Solving Forensic Cases

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**Learning Overview:** After attending this presentation, attendees will understand the role of video recording systems in forensic investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting six cases in which video systems were crucial for reconstructing the events.

To date, video surveillance systems guarantee safety both to people and items. Video surveillance systems have also changed over time, both in technology and in approach. The new generation systems allow sharp image details, and the advent of digital technology has made possible the development of so-called “video analysis.” This allows, through sophisticated software, the automatic recognition of any critical issues and different applications. Modern video monitoring systems are designed to perform multiple tasks simultaneously. From the legal point of view, it is important to extract the video recording to reveal the guilty party of a crime and provide evidence of guilt. In the forensic field, video recording systems are often useful even when they record in neighboring areas and become, in a completely random way, fundamental elements for solving cases since they are located in areas where crimes took place, then used for judicial purposes. The purpose of this study was to evaluate the role of video recordings in the analysis of scenes in which often the dynamics and events are not clear and present doubts about witnesses, statements, and culprits. In every case, a detailed scene analysis, an autopsy, and a video recording analysis were performed. In all cases, the video recordings were reproduced on CD-ROM support and were analyzed through the computer screen. Finally, all the video recordings were compared with autopsy findings and witnesses.

Reported are six forensic cases in which the acquisition of videotaped images was analyzed after the events. The first reported case is the death of a man who was hit by a car. The dynamics of the event were unclear and the driver’s story about the lack of road visibility aroused doubts. The analysis of the images clarified the driver’s responsibility. In the second case, a woman fell from the second floor of a building. In this case, it was not clear if the manner of death was suicidal or accidental. The analysis of the images allowed the visualization of the suicidal will of the woman. The third reported case was a case of fire that caused the death of two people. The manner of the fire was doubtful, in particular if it was intentional or malicious. The analysis of the video recordings made it possible to understand the involvement of the victims in the fire and the investigation revealed the motive. The fourth case concerns the death of a girl following a car accident. In particular, video recordings clarified the responsibilities of the accident and the speed of the vehicles involved. In the fifth case, the death of a woman due to a heinous murder is reported. Video recordings of cameras placed on a street made it possible to clarify the veracity of the testimony of a subject and his role in the murder. In the sixth case, the death of a girl in a psychiatric clinic was analyzed during lunch because of a choking. In this case, the intervention of the health personnel was doubtful and investigations about the correctness of the first aid were performed. Security camera recordings continue to play an increasingly important role in crime investigations. Visual information is very important independent of the fact that it concerns a crime or a criminal activity. However, investigators often only have recordings, taken without any forensic precautions, which are useless or rendered unusable. There are also limits due to the technical characteristics of the equipment used; the most frequent, for a subsequent analysis and elaboration, are low resolution and image frequency, camera shooting angle, inadequate lighting conditions, and incorrect or insufficiently documented time references.

It is necessary, for forensic purposes, that the preservation in the native format of the extracted videos avoids conversions in other formats that could further deteriorate the quality of the original images and cause the loss of the precious metadata that could be crucial for the reconstruction of the events. Therefore, it is essential to use video recordings as a tool for the complete acquisition of images, such as acquisition of evidence or clues about times, date, and people; clarification of dynamics; and exclusion or inclusion of any alibis.

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### Forensic Science, Technology, Software

## E62 A Mysterious Case of a Serial Killer in Italy: “The Monster of Florence”—Is the Case Really Solved?

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**Learning Overview:** After attending this presentation, attendees will know of a famous cold case about an Italian serial killer.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through a historical reconstruction of the event and by analyzing the criminological profile of the killer.

Serial murders are rare events. Scientific studies show that the most common reason that leads a serial killer to commit murder is the extrinsic gratification of the act. Psychopathic personality and experiences of child sexual abuse are common traits. The cases of various serial murders that occurred in Italy are reported. A survey was conducted about the proceedings of the trial, the analysis of Italian newspapers, and historical video recordings concerning the trial of the serial killer named “The Monster of Florence” who was responsible for 16 murders that occurred between 1968 and 1985, all in the Tuscan countryside near Florence, Italy. The target was always the same: young couples who were secluded for intimacy. The investigations revealed a constant ritual: the first aggression was always directed toward the man, and only later was the woman killed. All the crimes were carried out by using the same weapon: a Beretta .22 caliber also with Winchester® H series bullets. The murders that took place after 1974 were characterized by a further macabre element: the abuse of the corpses of female victims; in fact, the killer carried out a removal of the pubis and parts of the breast. This last element, given the surgical precision of the executing hand, suggested that the killer should be a person able to perform precise cuts, with the hypothesis that he would be a surgeon.

At the time of the facts, there was no standardized method in crime scene analysis, and the scene was not crystallized. Usually the first people who arrived at the crime scene were journalists. This resulted in a serious compromise of the scene, with the loss of important evidence for the purposes of investigations and with years of delay in identifying the real killer. The analysis of the judicial processes has highlighted several people convicted only according to circumstantial evidence and subsequently released because the monster continued to kill other couples during their imprisonment. After numerous investigations, the Court of Florence definitively condemned the so-called “Compagni di Merende” (Buddies of Snacks) Mario Vanni (deceased in 2009) and Gianluca Lotti (deceased in 2002), while Pietro Pacciani, first acquitted in the appeal process, died in 1998, before of the end of the trial.

The psychopathological profile of the “Buddies of Snacks” was very similar. The three had low socio-cultural backgrounds and were all indigent and chronic alcoholics with sexual perversions. In particular, Pacciani presented a story of sexual violence toward his daughters, victims of years of abuse, and a previous murder committed when he was young. Surely the three could be the material executors of the crimes for a series of clues evaluated in the process, but the presence of a mandate is assumed. Investigators found large sums of money in Pacciani’s home. No element attributable to symbolism, ritualism, or an obsessive pathology was found in the homes of the condemned. Then who is The Monster of Florence? The most accredited theory of the investigators is that there were one or more principals who manipulated the “Buddies of Snacks” who were weak men, easily controllable, and susceptible to blackmail through easy mental manipulation. The “monster” in fact has always adopted a rigid executive method: the murders always took place over the weekend; no gun or knife was found; there was never a rape of the victims; there were no traces of spermatic fluid; and the psychological element was focused on the certainty of killing the victims. The three dead convicts, for their psychological and behavioral background, would never have perpetrated their homicides in such a precise and constant manner, repeating these murders in a maniacally precise manner. Is the Monster of Florence really a solved case? It is believed not.

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### Forensic Science, Cold Case, Serial

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## E63 The “Tomato” Falsification of the Crime Scene: A Special Case of Murder

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**Learning Overview:** After attending this presentation, attendees will understand the role of autopsy in apparent natural deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the role of the depth of forensic analysis in cases of chaotic scenes involving the presence of numerous confounding elements.

Crime scene analysis is essential in resolving forensic cases. The forensic pathologist can be found in open spaces or closed places, and the analysis of trace evidence is often influenced by external factors. It is increasingly difficult to find trace evidence, especially when the scene is contaminated. In particular, it can happen that the scene is falsified in different ways and sometimes the investigators are led to incorrect forensic conclusions about false clues, false suspects, and false trace evidence. In these cases, it is important to use a unique operating protocol to avoid judicial errors.

This study reports the case of a person of Romanian nationality, a known chronic alcohol user, found dead in his own home. The scene was photographed from the periphery to the center (intended as the point of discovery of the corpse). An external examination of the corpse with the surveys of the postmortem interval was carried out. The analysis of the scene showed poor hygienic and sanitary conditions with bottles and food residue scattered around the house with glass from broken bottles and a strong smell of alcoholic substances. The body lay between the floor and the bed, lying on the floor. The whole scene was completely sprinkled with tomato sauce so it was difficult to identify the presence of important trace evidence for the investigation. The body was analyzed and externally it seemed to show no signs of injuries except for a small cut on the eyebrow. Investigators wanted to promote the removal of the body without performing an autopsy, presuming a trivial case of natural death in a domestic environment. Nevertheless, an autopsy was conducted.

Externally the corpse appeared completely covered with tomato sauce so that, before the autopsy, the clothes were dried and preserved. Only after washing did the skin show widespread ecchymosis all over the body and on the head. In particular, there was a large thoracic and abdominal hemorrhage due to blunt object trauma. The internal examination showed widespread hemorrhagic outcomes at the level of the head with a homicidal mode. The musculature of the neck and chest showed widespread crushing hemorrhages and signs of manual strangulation. Multiple and bilateral rib fractures and hemothorax were found. Abdominal organs showed widespread signs of lacerations. All the injuries found at autopsy were not compatible with the absence of data detectable by the analysis of the falsified scene. The death of the man was due to cardiorespiratory failure secondary to head trauma, thoracic crush trauma, and laceration of internal organs, with manual strangulation in a subject under the effects of alcohol. In scenes where confounding elements are present, the analysis of all the evaluable elements and the use of standardized methodologies is fundamental.

In particular, it is suggested that an autopsy be conducted on subjects found dead in the absence of obvious external injuries. Blunt injuries are easily falsifiable externally due to the intrinsic characteristics of the weapon used and to the local harmful effects in the absence of external lesions. Furthermore, it is pointed out that often an attempt at manual strangulation may not cause external signs on the neck that are visible during a simple inspection. This depends on various factors such as the means used, the force, and the duration and the dynamics of the event. In particular, in the presence of attempts to falsify and conceal evidence in order to falsify the cause of death, an autopsy is crucial to analyze apparent accidental or natural deaths. In doubtful cases in the absence of evident external injuries, the autopsy is essential, especially when it comes to enclosed spaces in apparently contaminated scenes. In this case, if information was limited only to the visible elements of the crime scene, the investigation would not have continued. The case would have been archived as a natural death and the murderer would have gone unpunished. In this case, full of confounding elements, all the limits of the judicial inspection emerged, especially since the crime scene had been contaminated with the aim of confusing the investigation. The autopsy is, and will remain, an essential element in the reconstruction of a criminal act in chaotic and falsified crime scenes with multiple variables.

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### Forensic Sciences, Falsification of Crime Scene, Murder

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## E64 A Penetrating Chest Trauma Due to a High-Velocity Projectile in the Workplace

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**Learning Overview:** After attending this presentation, attendees will be aware of the various fatal injuries that can occur due to freak accidents at the workplace involving high-velocity projectiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the injury profile of penetrating traumatic injuries at a construction site.

Penetrating trauma is that which is caused by a foreign object piercing the skin, damaging the underlying tissues, and leading to an open wound. In addition to Gunshot Wounds (GSWs) and stab wounds, these injuries include other types of impalements as well and can range in severity from superficial punctures to penetration of major body systems, including vital organs, that may lead to the death of the person. Thoracic lesions are a major aggravating factor in patients with multisystem trauma, accounting for nearly 20% of all deaths of penetrating traumatic origin. There are very few reported deaths due to penetrating projectiles, and penetrating injuries over the chest are even more rare. It is a known fact that mechanical injuries in a factory or construction site set up are common occurrences in India, although they are not always fatal. They can be attributed to a lack of precautionary and safety measures. Occupational injuries from projectiles are common at workplaces, but they are not usually fatal as they only affect the eyes or subcutaneous tissue.

In this case, an incidence is reported where a part of the machinery acted like an unusual projectile, leading to devastating chest injuries causing death. Most of the time, gross injuries may be visible, but in certain cases there may be minimal external injury. Crime scene examination led to the reconstruction of the exact cause and circumstances of death. This case brings forth the already understood penetrating trauma aspects of wound ballistics that can be used to explain the response of all tissue to penetrating trauma of all types, thereby helping to predict and explain the severity or lack of severity of tissue injury in trauma in general. While there are several cases about penetrating injuries due to unusual projectile foreign bodies, no cases have been discussed in recent literature, per this study's research. Proper evaluation requires crime scene correlation and comparison of the metallic fragment or projectile retrieved at autopsy and machinery and tools from the scene of the incident.

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### Penetrating Chest Injury, High-Velocity Projectile, Occupational Injuries





**E65 Comparing Illicit Opioids and Synthetics Collected From Death Scene Investigations to Toxicology Results of Overdose Deaths**

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**WITHDRAWN**

## E66 An Unusual Suicide With Multiple Stab Wounds

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**Learning Overview:** After attending this presentation, attendees will understand the role of the differential diagnosis between homicide and suicide in cases of multiple stab wounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of comparing the data at the crime scene with the analysis of the topography of the external and internal injuries found at autopsy.

Stab wounds are injuries produced on the tissues by pointed and/or cutting instruments. Statistically, the finding of stab wounds on a corpse, especially when they are multiple and located in different topographical areas, is attributable to a homicide. However, to avoid potential errors with important judicial repercussions, before providing a definitive judgment on the event, the coroner has the duty to assess the possibility of a self-injurious event through an accurate differential diagnosis. Described here is a rare case of suicide with a stab weapon in which numerous injuries were found. Only the careful comparison of the scientific evidence that emerged from the crime scene investigation, the autopsy, and the histopathological investigations allowed the reconstruction of the real manner of the event.

A male corpse was found inside the bathroom of a bakery located in the Municipality of Vauda Canavese (Turin, Italy). The coroner carried out an initial judicial inspection at the crime scene and described the presence of a sharp weapon (kitchen knife) in the left thoracic region, the presence of numerous puncture and cut injuries of various depths to the anterior region of the neck and in the left hemithorax. The judicial inspection coordinated by the Operations Department of the Scientific Investigations Section in aid of forensic doctors called by the judicial authority allowed the description of the state of the places with greater attention and verified that the blood stains were present only on the floor and on the walls of the bathroom, with particular attention to the portion of the bathroom where the corpse was found, in the absence of directed projections from/toward the entrance door of the room. The forensic pathologist performed an autopsy and described 22 oval puncture and cut injuries penetrating the skin and subcutaneous tissues externally in the anterior cervical region and about 20 stab and cutting wounds in the left precordial and axillary region. At the internal examination, with the knife removed, a non-penetrating wound in the chest was observed, nine continuous solutions from the tip and a cut through the left lung on the anterior and posterior side with abundant blood into the left pleural cavity. During the autopsy, fragments of various organs were taken for histological investigation. A urinary screening examination was carried out using the triage method for the main substances of abuse with a positive result for benzodiazepines. Second-level toxicological investigations conducted on blood confirmed the intake of benzodiazepines (diazepam) and its metabolites (nordiazepam, oxazepam, and temazepam). The data collected with the medicolegal technical investigation of specific competence, together with the circumstantial data, made it possible to conclude that the man died following a hemorrhagic shock from multiple tip and cutting injuries in the neck and in the precordial region resulting from an unusual suicide. The positivity for psychotropic drugs was probably attributable to the voluntary intake of drugs by the man in order to strengthen his intent.

The case reported demonstrates how important it is to make an accurate differential diagnosis before ruling out suicide. For this purpose, it is suggested to carefully evaluate the areas drawn. Usually, the suicide by swab weapon is carried out by drawing on the precordial region, the neck region, or the epigastric region, and the blows are inflicted with denudation of that skin area.

In homicide, however, it is much more frequent that the affected areas are random, due to the excitement of the event and the high probability of a fight before death. For this reason, it is essential to carefully investigate the presence of active and/or passive defense injuries attributable to an attempt to defend oneself by the victim. For this purpose, this study suggests investigating the presence of active defense wounds on the palms of the hands (in an attempt to block the blade), passive defense wounds on the back of the hands (in an attempt to defend against inflicted blows), and dodging wounds (caused by attempting to evade blows). The case reported demonstrates that the number of blows inflicted, even if numerous, is not sufficient to exclude suicide with certainty, because it is possible that the victim also self-inflicted multiple blows in an attempt to die, as in this case.

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### Forensic Sciences, Suicide, Stab Wounds

## E67 The Importance of an Interdisciplinary Approach for a Differential Diagnosis of Child Abuse: A Case Report

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**Learning Overview:** After attending this presentation, attendees will understand how, in the absence of a history of trauma or medical predisposing conditions, physical abuse should be considered as a differential diagnosis when children present cutaneous bruising or other bleeding manifestations, abrasions, and fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that an interdisciplinary evaluation and a holistic approach are indispensable for the prevention and the diagnosis of Child Abuse (CA).

CA represents all forms of physical and/or emotional ill treatment, sexual abuse, neglect, and exploitation resulting in actual or potential harm to the child's health, survival, development, or dignity in the context of a relationship of responsibility, trust, or power.<sup>1</sup> CA represents a serious problem and the misdiagnosis or lack thereof is responsible for grave consequences with a 10% mortality rate and a 50% reinjury rate.<sup>2</sup> The diagnosis of CA is often an intuition of physicians. However, suspicion is not evidence and, therefore, health care workers should be able to recognize medical conditions that can simulate child maltreatment<sup>3</sup> and alerting features.<sup>3</sup>

Reported here is an interesting case of a 3-month-old baby hospitalized by the parents due to the appearance of hematomas. A thorough medical history and a careful physical and clinical examination allowed physicians to rule out a hematological disease and to hypothesize CA. Therefore, they promptly transferred the child to the emergency room where the physicians could confirm the presence of abrasions on the face, a crusty lesion on the right nostril, a bruise on the left arm, the presence of a hematoma in the right shoulder with pain on mobilization. Skeletal Survey (SS) and thoraco-abdominal Computed Tomography (CT) investigations were also performed with evidence of multiple bilateral rib fractures with a discrete callus formation and lateral middle third right clavicle fracture with no callus evidence. The brain CT showed no signs of hemorrhage. The child was also subjected to a negative abdominal ultrasound, an eye examination with findings of right subconjunctival hemorrhage and some scratching eyelid lesions. Therefore, suspecting child maltreatment, the police were alerted and the public prosecutor disposed a forensic consultation.

The forensic investigation was performed and confirmed the presence of abuse signs: widespread bruising on the face, left cheek, and left upper limb with a bite mark on the left forearm, a second-degree abrasion in the anterior neck region, a right nasal fold excoriation, and right eye conjunctival hemorrhage. The radiological investigation findings and the skin lesions analysis and distribution provided useful information to define the manner and age of the lesions.

A precise assessment of bruises, based on evaluation of chromatic changes in subcutaneous hemorrhages, of the injuries multipolarity and bone fractures made it possible to assert that the injuries were not correlated and backdated to a single episode but to more than one violent traumatic episode. These injuries were compatible with a blunt injurious suitability that acted both with a compressive mechanical action of grasping and crushing and with an abrasive tangential concussive action, detected by the presence of a right sub-conjunctival hemorrhage that is considered a "marker" in recognizing young shaken baby syndrome.<sup>4</sup> The forensic investigation confirmed that injuries were compatible with a child abuse diagnosis.

An interdisciplinary evaluation and a holistic approach are fundamental for the prevention and the diagnosis of child abuse.<sup>5</sup> When children present with cutaneous bruising or other bleeding manifestations, abrasions, and fractures, in the absence of a history of trauma or other predisposing medical condition, physical abuse should be considered in the differential diagnosis.<sup>6</sup> Bruises and fractures are the most common injuries found in physical abuse; therefore, a thorough forensic investigation including the assessment of the characteristics, the extent and type of bruising, and the skeletal survey<sup>9</sup> appear to be the first line of investigation for suspected physical maltreatment.<sup>7-9</sup>

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## Forensic Sciences, Child Abuse, Maltreatment

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## E68 Infant Homicide: Perpetrators, Causes, and Prevention

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**Learning Overview:** After attending this presentation, attendees will have learned the most likely perpetrators of infant homicide, the associated risk factors, and potential prevention measures to increase infants health and safety.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by providing critical information concerning a high-risk group of medicolegal death investigation and by increasing awareness of infant homicide and possible prevention strategies to avoid infanticide.

The prevalence of infanticide in the medicolegal death investigation is an underresearched area within the forensic community. This research aims to focus on infanticide's critical aspects, such as the most likely perpetrator, associated risk factors, and potential prevention measurements. Utilizing autopsy records from the Office of the Chief Medical Examiner (OCME), a retrospective study was conducted examining 41 cases of infant homicide over 19 years dating between 1999 and 2018.

Infanticide is defined as the intentional killing of an infant greater than one day and less than or equal to 12 months of age caused by a parent, guardian, or caretaker.<sup>1</sup> This comprehensive study revealed that of the 41 infanticide cases, the biological parents were most likely perpetrators. The mother was the most common perpetrator (32.4%), followed by the father (24.75%), the mother's boyfriend (16.4%), an individual unrelated to the child (8.36%) in shooting or house fire by arson, and a babysitter (6.02%). The most common causes of deaths were asphyxia (38%) and head injuries as a result of blunt force trauma (38%), followed by multiple varying injuries (12%), burn/thermal injuries (5%), gunshot wound (3%), sharp force injuries (2%), and methadone intoxication (2%).

The most common victims were White (48%), followed by African Americans (40%), Hispanic (10%), and Asian (2%). Males were disproportionately affected at 62% in comparison to females at 38%. The age group for victims ranged from 1 day to 12 months, with the highest frequency of reported deaths being infants 1–2 months of age (55%). This increase in frequency suggests that infants within 1–2 months of age have a higher risk of becoming homicide victims. While contributing factors range from family stress, availability of resources, and cultures of violence, most infanticides occurred due to sudden anger or rage from the caregiver as a result of the baby's crying.

The risk factors of infanticide include: (1) caregiver/family risk factors such as young, single, poor, unemployed parents, less education, low self-esteem, poor control of their impulses and mental health issues, a history of drug or alcohol abuse, domestic violence, or lack of social support; and (2) community/society factors such as a high crime rate and high poverty rate. The highest infanticides occurred in Baltimore, MD, which has the highest crime rate and poverty rate in Maryland. Prevention efforts should focus on socioeconomic support to the new parents, education programs to teach caregivers how to control their emotions and deal with infants' crying and their needs, and community crime-reduction programs.

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- <sup>1</sup>. Brewster, Albert L., John Nelson, Kent Hymel, Donald R. Colby, Don R. Lucas, Thomas R. McCanne, and Joel S. Milner. Victim, Perpetrator, Family, and Incident Characteristics of 32 Infant Maltreatment Deaths in the United States Air Force. *Child Abuse & Neglect* 22, no. 2 (1998): 91-101. [https://doi.org/10.1016/S0145-2134\(97\)00132-4](https://doi.org/10.1016/S0145-2134(97)00132-4).

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### Infant Homicide, Risk Factors, Prevention

## E69 The Importance of Postmortem Investigations in Intrauterine Fetal Death: Case Studies and a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will know why the fetal autopsy and placental examination should be common practice in studying the causes of intrauterine fetal deaths. The only maternal and gestational clinical history in most cases is not enough to determine the cause of death and today many cases are still unanswered. The forensic pathologist, therefore, has an essential role in the management of intrauterine fetal deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the purpose of postmortem investigation is to determine the causes of death and its mechanisms, as well as to help both health care professionals and parents understand the complex dynamics of intrauterine fetal death by reducing the number of unexplained cases and thus manage future pregnancies. For this reason, the results obtained by the forensic pathologist must always be integrated with clinical information in order to have a complete picture so as to identify the cause of the unfortunate event.

The World Health Organization (WHO) defines intrauterine fetal death as the loss of the fetus after the 22<sup>nd</sup> week of gestation or, in the case of unknown dating, for a birth weight greater than or equal to 500 grams. Intrauterine fetal death is one of the most frequent adverse events in pregnancy and contributes to a large part of perinatal mortality with an important economic and social impact. Stillbirth complicates 6 per 1,000 pregnancies in the United States, or approximately 1 in 160 pregnancies.<sup>1</sup> A variable but important percentage (15%–60%) of stillbirths still remain unexplained today.<sup>2</sup> The purpose of this study is to quantify the contribution of the postmortem and placental examinations in identifying the cause of intrauterine fetal death.

Reported here is the analysis of 11 cases of intrauterine fetal death carried out through a retrospective study conducted in the period between 2014 and 2017. For each case, this study proceeded to analyze the medical record with the relative maternal and partner data, the results of the external fetal and autopsy examination, as well as the macroscopic and histological placental examination. Fetal autopsies and placental histological examination were performed using a standard protocol. The average maternal age was 30.4 years; 63.6% of intrauterine fetal deaths were in the age group between 30 and 39 years. One or more maternal risk factors were present in all cases examined (weight gain, 54.5%; cigarette smoking, 22.2%; diabetes mellitus type II, 22.2%; arterial hypertension, 11%). The risk of stillbirth increased with gestational age; 72.8% of cases occurred after the 36<sup>th</sup> week and no cases occurred before the 29<sup>th</sup> week. Diseases of the umbilical cord, placenta, and amniotic fluid were responsible for 90.9% of the cases of intrauterine fetal deaths. The results gained from the clinical history and external fetal examination made it possible to track the cause of death only in 18.2% of the cases. By adding to these data the results of the fetal autopsy and placental examination, it was possible to establish the cause of death in 90.9% of cases; 9.1% of intrauterine fetal deaths remained unexplained. This study also highlights how external fetal examination only would have led in most cases to a wrong diagnosis of death. Accurate analysis of postmortem placental and fetal examination is essential to reduce the number of unresponsive intrauterine fetal deaths. Advances in histo-pathological and forensic science will make it possible in the future to eliminate the percentage of still-unexplained stillbirths. Linking clinical and histopathological examinations is essential to understand what happened during the gestational period and to prevent a similar outcome in a subsequent pregnancy.

### Reference(s):

1. MacDorman, Marian F., Uma M. Reddy, Robert M. Silver. Trends in Stillbirth by Gestational Age in the United States, 2006–2012. *Obstetrics and Gynecology*. 126, no. 6 (2015): 1146–1150.
2. Gardosi, Jason, Sue M. Kady, Pat McGeown, Andre Francis, and Ann Tonks. Classification of Stillbirth by Relevant Condition at Death (ReCoDe): Population Based Cohort Study. *BMJ* 331, no. 7425 (2005): 1113–1117.

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### Forensic Sciences, Stillbirth, Forensic Autopsy

## E70 Comparing Virtual Tools for Investigator Training

*Katherine Ramsland, PhD\*, DeSales University, Center Valley, PA 18034*

**Learning Overview:** After attending this presentation, attendees will better understand different types of extended reality platforms and their facility for building models for investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the benefits and drawbacks of using a virtual platform for teaching crime investigation.

The concept of extended reality encompasses all forms of augmentation or digital representation in a virtual arena.<sup>1</sup> Augmented Reality (AR) blends interactive digital elements, such as overlays and sensory projections, with real-world environments. Using a device like a smart phone camera, viewers can project game items that look like part of the physical environment.<sup>2</sup> The United States Army uses AR tools to digitally enhance training missions. Virtual Reality (VR) is a 3D computer-generated environment with which one can interact as a participant in the virtual world, manipulating virtual objects. Participation requires a VR device, such as headset with a display and headphones.

Extended Reality (ER) has moved into forensics.<sup>3</sup> Using AR and VR tools, investigators can generate different perspectives to enhance their ability to make effective decisions about a crime scene, and students can learn how to investigate without corrupting it. The virtual recreation of a crime scene has potential uses for teaching in a course, a police training, and court demonstrations. In VR, investigators can modify the scene to remove irrelevant objects for easier observation of evidence or add objects as red herrings.<sup>4</sup>

Most crime investigation trainings dispense information rather than offer hands-on experience, because they cannot use actual crime scenes. With the development of ER platforms, students and police officers gain opportunities to experience realistic scenes. However, the technology can be confusing and expensive, which leaves this approach underutilized. Comparative research with ER methods can help to identify the most accessible and affordable approach.

A Forensic Sciences Foundation (FSF) Lucas grant enabled a team to compare two methods in a virtual scene. The team rebuilt the 1971 John List familicide scene in New Jersey from crime scene photos and actual house dimensions on Linden Lab's Second Life gaming platform.<sup>5</sup> They set up a Roar AR app to use avatars to view the items inside. Approaching a body, for example, they could activate the app and read the autopsy report. Using the app on a note that List wrote, they could hear him reading it. They also tried out the Unity gaming platform.

The VR method used untethered Oculus® Quest headsets, which place wearers inside a vivid 3D, 360-virtual environment. They could move through a scene as if walking and use virtual hands to touch and grab things. In a virtual crime scene, participants could look at evidence from different angles and devise a reconstruction.

The team showed their work to a local coroner who runs training sessions and discussed the pros and cons of both methods in terms of cost and accessibility. The consensus was that a recent upgrade to the Oculus® headset made it the best tool to couple with a Second Life/Roar combination, but while cost-effective, the helmet still had key limitations.

### Reference(s):

1. Bailenson, Jeremy. *Experience on Demand: What Virtual Reality Is, How it Works, and What It Can Do*. New York: Norton, 2018.
2. M. Bower, C. Howe, N. McCredie, A. Robinson, and D. Grover Augmented Reality in Education – Cases, Places and Potentials. *Educational Media International* 51, no. 1 (2014): 1-15.
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### Augmented Reality, Virtual Reality, Digital Crime Scene Investigation

## E71 Pushing the Bounds of Virtual Education: The Creation of an Interactive, Virtual Forensic Serology Lab

*Catherine Cupples Connon, PhD\*, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** The goals of this presentation are to: (1) familiarize attendees with an interactive, virtual forensic serology laboratory powered through Microsoft® PowerPoint®; and (2) demonstrate that effective virtual laboratories can be prepared at low cost and easy student access to replace and/or supplement in-person laboratories when necessary.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a virtual option for forensic courses, whether due to willingly creating an online course, responding to an emergency situation requiring virtual coursework, or even accommodating a student who will be absent from class. Additionally, this interactive forensic serology laboratory could be used as part of a training tool for crime laboratories.

Virtual courses became the norm for many students in the spring and fall of 2020 due to the COVID-19 pandemic. Educators faced numerous challenges: maintaining rigor and student interest, preventing cheating, and even protecting their intellectual creations. Furthermore, resources were scarce, and time was limited. What would normally take months to prepare had to be prepared in a fraction of that time. This presentation will provide a thorough demonstration of the virtual forensic serology lab, including development, challenges, and student feedback, which was overwhelmingly positive.

The virtual lab included a combination of enhancement, presumptive, and confirmatory tests for blood and semen, which included some historical and current analyses common to the forensic community. Forensic serological tests for blood included luminol, fluorescein, combined Phenolphthalein Tetramethylbenzidine (P-TMB), Takayama, and ABACard® HemaTrace®. Forensic serological tests for semen included long wave Ultraviolet (UV), Alternate Light Source (UltraLite ALS®), acid phosphatase, ABACard®p30, and sperm search. Development via Microsoft® PowerPoint® relied on the use of detailed and extensive animations triggered by students clicking on equipment, reagents, and/or samples. Though participants needed to follow along in their lab manual for step-by-step instruction, on-screen prompts were also used to remind students to disinfect their benchtop and utensils, process controls, etc. Student access to the virtual lab was fairly simple: download and extract a ZIP file, then open the virtual lab in PowerPoint® Slide Show (.ppxs) mode. Failure to extract the ZIP file was the most common error and resulted in the inability to correctly access hidden files via embedded hyperlinks. The virtual lab was also compatible using both PC or MAC®, but was easier to access via PC than MAC® because all files were hidden on a PC except the .ppsx files. On a MAC®, all files intended to be hidden were visible to the student, and this led to confusion regarding which file(s) to open.

Though this virtual lab was prepared for the worst-case scenario, in-person labs were in fact carried out in fall 2020, and this virtual lab was therefore used as an effective supplement to the in-person lab, as well as a supplement to those that were enrolled in serology lecture only. Both undergraduate (22) and graduate students (21) enrolled in a forensic serology course at Virginia Commonwealth University had access to this virtual lab. Whether enrolled in an in-person forensic serology lab or lecture only (no lab), more than 90% of the surveyed students indicated that they found the virtual lab effective to further their understanding of body fluid analysis; the actual testing procedures; general precautions for contamination prevention; processing controls; and following a standard operating procedure. Additionally, those that were enrolled in the in-person lab indicated that the virtual lab was very similar to the hands-on lab. Based upon these results, it has been demonstrated that effective virtual laboratories can be prepared at low cost and easy student access to replace and/or supplement in-person laboratories when necessary; however, it is ideal to maintain the in-person experience.

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### Education, Virtual Lab, Serology





### **E72 Assessing the Impact of Using Virtual Reality as a Training Tool Compared to Role-Play Simulation in the Forensic Management of the Dead Training**

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**WITHDRAWN**

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## E73 Create Virtual Tours Using Google® Tour Creator for Remote Instruction

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**Learning Overview:** The goals of this presentation are to: (1) examine the use of virtual tours in online instruction; (2) describe the benefits of virtual tours in a learning environment; and (3) create a virtual tour of spaces to promote learning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by developing an understanding of how virtual tours and field trips can be used in forensic science education. This presentation will allow attendees to learn the benefits of virtual tours as well as see examples of how virtual tours are created. This will impact the forensic education community by teaching how to create remote learning experiences for learners and how implantation of virtual tours can be used as a learning experience.

A part of the learning experience is being able to physically see places, whether a geographic location, historical buildings, or a working environment. Having the ability to take students on field trips is a popular learning tool used in the K-12 school system, but why not in higher education or training programs? Usually, there are multiple restraints such as space limitations, travel and location, or other factors. However, there is a way to create virtual field trips and tours of locations for students. Students can benefit from seeing a real forensic laboratory, seeing first hand the instrumentation used in the crime lab, different units of the lab, and safety features of the lab. This project started for online learners because of the non-presence in a laboratory setting. Students needed to be exposed to safety features in the laboratory as well as waste management and common equipment and tools used in the lab. However, this can be applied to all learning environments as well as a marketing tool.

Two separate forensic labs in Indianapolis, IN, were visited by only the instructor and instructional online course designers. Photographs of various lab units were taken with a 360° camera as well as still photographs of equipment, specific features of the lab space, and tools used in evidence analysis. Using the Google® Tour Creator program, virtual tours of both the Indiana State Police Crime Lab and Indiana State Department of Toxicology Lab were created. This has created a multi-dimensional experience for the learners. Each tour includes many scenes that lay out the lab space. Within each scene, specific areas in the lab or instrumentation used in evidence analysis is highlighted with close-up photographs and descriptions. Students are able to go through the forensic laboratory space to see the equipment and learn about how that piece of equipment is used in forensic analysis.

This tool can also be used to highlight educational space and create virtual tours for potential students. Highlighting the instrumentation, lab space, and equipment is a great marketing tool that can be used to show what universities have to offer. Many potential students want to see classrooms and laboratory spaces before deciding on where to attend college as well as graduate students wanting to see the research lab space. Travel may not be possible for students from a greater distance, and this will provide a way to show potential students teaching and learning spaces.

Throughout this presentation the ways in which virtual tours of local crime laboratories and teaching laboratory space were created will be shared. A simple use of photographs and descriptions were used to create a virtual learning experience for students. This is used as a learning tool in online courses where students are asked to identify equipment and learn the use of various tools in the lab. Just because traveling to a location cannot happen does not mean that students cannot visit a real crime laboratory.

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### Forensic Education, Virtual Tours, Online Learning



## **E74 Innocence Project Initiative and Cold Case Program—Experiential Applications of Constructivism for Unbiased Investigative Public Service**

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**Learning Overview:** After attending this presentation, attendees will better understand how initiatives and programs can be developed at the post-secondary level to provide extramural, collaborative opportunities in the experiential applications of constructivism and cohort-based case analytics and how these efforts provide for the practical incorporation of the sentinel concepts of objective investigational justice and judicial equality into the forensic science educational curriculum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying course-based efforts at the post-secondary level that exemplify a balanced, holistic, and highly unique educational integration of object forensic science and unbiased investigative public service.

The W. Roger Webb Forensic Science Institute Innocence Project Initiative and Cold Case Program provide extramural, collaborative opportunities in the experiential applications of constructivism and cohort-based case analytics for students, faculty, and judicial practitioners. These course-based efforts exemplify a well-conceived, balanced, holistic, and highly unique educational integration of object forensic science and unbiased investigative public service. Both courses utilize authentic investigative milieus, centered in a focused, systematic, multi-disciplinary examination of unsolved violent crimes and unresolved issues in forensic evidence recognition and analyses, to develop learning experiences that are contextualized and active. These collaborative endeavors provide dedicated forensic science support and analytic expertise to substantive community service programs frequently lacking in adequate consultative resources. Student learning is regulated, supervised, and mediated through complex integrated, individual and group critical thinking and problem solving. Students are tasked with reviewing actual case files and utilizing conceptual, structural, and experiential knowledge to make scientifically sound judgements and recommendations concerning the application of appropriate, relevant forensic science concepts and analyses in the furtherance of potential investigative leads and judicial illumination. Learners are required to use high-level cognitive skills, decision-making schema, and productive modes of inter-personal communication to provide actionable results and implementations of their findings.

The goals of the Innocence Project Initiative and Cold Case Program are to prepare students to professionally and ethically evaluate complex cases, conduct focused forensic research, and develop novel, previously unexplored investigational avenues and strategies. These efforts provide for the practical incorporation of the sentinel concepts of objective investigational justice and judicial equality into a forensic science educational curriculum.

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### **Innocence Project, Cold Case, Investigative Public Service**



## E75 Enriching the Forensic Science Curriculum Through a Guided Study Abroad Experience

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**Learning Overview:** After attending this presentation, attendees will understand how the forensic science curriculum at both the undergraduate and graduate levels can be enhanced through a forensic science learning abroad class and trip.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering an educational model that can both enrich students' perspectives of their chosen fields and expose them to alternative systems of criminal justice and forensic science practice.

Study abroad opportunities are a feature of most higher education institutions in the United States. Students can choose to spend a semester or even an entire year at a foreign university, usually during the student's Junior year. According to the Institute for International Education, about 16% of all students earning a bachelor's degree will study abroad at some point.<sup>1</sup> However, some students see a full semester abroad as too much of a commitment, especially for those with demanding or highly structured major requirements. More desirable are short-term trips lasting one week to ten days that are designed around a specific theme or topic. These trips are facilitated by faculty members and include an on-campus component prior to, and sometimes after, the travel component. More universities are implementing such study abroad programs targeting the Spring semester in order to utilize Spring break for the planned trip. Students and, more importantly, parents see short-term study abroad trips as safer as they tend to be heavily scheduled with little free time. For faculty, the ability to rely on an educational travel agent reduces planning responsibilities. Some organizations also provide tour guides, removing some of the supervisory burden from the traveling faculty member(s). After attending this presentation, attendees will learn how the forensic science curriculum at both the undergraduate and graduate levels can be enhanced through a forensic science learning abroad class and trip. This presentation will address the learning goals of a forensic science study abroad class, how to plan the international component, whether to engage with an international education provider, and how to deliver the on-campus course content. A study abroad class called "International Perspectives of Forensic Science" was designed and executed at Arcadia University in 2016. The trip took place over two weeks in May and included site visits in both Scotland and England. In 2018 it was adapted to fit into the Spring Break format at Rutgers University-Camden. Both versions of the trip will be compared and recommendations regarding duration, content, budgeting, and other logistical considerations will be presented.

The next generation of forensic scientists has the opportunity to improve the profession through leadership and creative problem-solving. By broadening students' outlook through international experiences, educational programs can guide students toward more innovative thinking. Study abroad also provides faculty with the ability to network with international counterparts, leading to additional exchange and research prospects.

### Reference(s):

- <sup>1</sup> Baer, Julie. Open Doors 2018: Report on International Educational Exchange. Institute of International Education, 2018.

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### Education, International, Curriculum

## E76 Setting a New Standard in Access and Quality for Forensic Science and Justice Training

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**Learning Overview:** The goal of this presentation is to provide a proven working solution to answer real-world needs and push forensic science and justice forward.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with an example of a working connection between laboratory practitioners and their academic researcher counterparts to increase their ability to identify a needs gap in methods, training, and equipment development.

There exists a siloed barrier between scientific research, real-world laboratory conditions, and practical field applications of technology. These worlds are often viewed as mutually exclusive, when, in fact, there is a deep-rooted need for a functioning symbiotic relationship. Researchers and laboratory practitioners often do not reach out to each other for collaboration in development of methods, training, and equipment beneficial to forensic science analysts in the cause of criminal justice. Working laboratories have the real-world experience to address the needs gap and offer possible working solutions. Research laboratories have the resources, including time and funding, to evaluate these new solutions. Field practitioners have the environmental experience to inform the development of useful technologies.

This forensic science and justice initiative brings together existing interdisciplinary collaborations to address discrepancies in forensic science practice and training and organize opportunities for practitioners and agencies to partner with researchers to solve real-world analysis and justice equality problems. By creating a relationship between laboratories and new technology development, this initiative encourages discussion on improving forensic science practice beyond the bench and courtroom.

We seek to expand experiential learning opportunities across forensic and traditional science disciplines, not just those in the traditional laboratory setting. The most recent partnerships include: (1) a program to serve regionally underserved Sexual Assault Nurse Examiners (SANE); (2) a microwave-accelerated DNA extraction method to speed investigative rapid DNA testing on both standard and challenging samples; (3) research to enhance public safety through effective identification, investigation, and prosecution of anti-Lesbian, Gay, Bisexual, Transgender, Questioning, Intersex, and Gender Non-Conforming (LGBTQ/GNC) hate crimes; (4) connections between environmental forensics and public health; (5) the creation of a new sampling device for ignitable liquid residue, or the evidence left behind at the scene of a fire, for use in investigations; (6) a two-year project with four metropolitan prosecutor's offices to improve data and analytical capacity, develop and implement new performance indicators to assess progress over time, and respond to the need to ameliorate racial and ethnic disparities in the justice system; (7) advancement of detection K-9 programs with state law enforcement; (8) testing to improve juror comprehension of forensic testimony and its effects on decision-making and evidence evaluation; (9) new digital information forensic course development and instruction for criminal investigators; (10) delivery of forensic training of effective evidence collection techniques to non-scientific practitioners in advanced locations in service of international courts of law; and (11) application of proven eye-witness recall techniques to improve COVID-19 contact tracing methods.

By building these cross-cutting relationships, we provide direct communication between practitioners and researchers—breaking down the previously held silos—to push forensic science training to higher levels of quality.

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### Training, Research, Innovation



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## E77 An Evaluation of a Model for Providing Cost-Effective, Accessible Continuing Education Content to Forensic Scientists

*Thomas J. Gluodenis, Jr., PhD\*, Lincoln University, Lincoln University, PA 1935*

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**Learning Overview:** The goal of this presentation is to present an updated model and its subsequent assessment for the development and optimized delivery of online continuing education for the forensic science community

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing best practices learned through the development and delivery of cost-effective, accessible, continuing education content approved by the American Board of Forensic Toxicology (ABFT) and the American Board of Criminalists (ABC) to more than 6,000 forensic students and professionals worldwide in an online format. Key learnings regarding the optimal event format, finance, technology, and operations will be shared.

Adequate training and continuing education are critical to ensuring the quality and credibility of forensic science practice.<sup>1</sup> Certification programs such as the one administered by ABFT and ABC have been applauded and recognized for their rigor in establishing educational, training, and experience requirements for forensic science professionals. A gap exists, however, in an individual's ability to identify cost-effective, accessible continuing education content to meet these certification requirements in as much as there is no programmatic approach to educational needs assessment and content development, the delivery channel (face-to-face, online, hybrid) is not optimized, and there is no central repository of available educational opportunities. As a result, not all forensic scientists are getting cost-effective access to the educational content required to remain current in their field. Online continuing education is on the rise as this modality of dissemination allows for accessibility, convenience, and affordability. This study presents a detailed model and its subsequent assessment for the development and optimized delivery of compelling online continuing education content for the forensic science community. The model, which includes an educational needs assessment, gap analysis, delivery and archival, along with participant feedback, was tested through the launch of an online symposium series, offered free of charge to participants. Each day, representing a virtual Master Class, an in-depth examination of a topic is presented by world-class practitioners having a unique mastery of the subject. A three-year study of over 6,000 participants representing >70 countries was undertaken to assess and improve the efficacy of the model relative to its stated goals.

Best practices were identified relative to organizational structure, technology, contingency planning, operational execution, and financing of the event along with opportunities to further enhance the model to meet the stated objectives of accessible, compelling, cost-effective continuing education for forensic professionals. This study provides empirical support for the use of online continuing education to support the professional development needs of the forensic science community. The results demonstrated that participants found the online symposium model fulfilled expressed learning goals and their engagement in the forum met required continuing education requirements.

### Reference(s):

- <sup>1</sup>. National Research Council (NRC) Committee on Identifying the Needs of the Forensic Science Community. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press; 2019: 29.

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### Continuing Education, Online, Virtual

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## E78 Feeling Stress at Work? Stress, Support, and Decision-Making of Forensic Examiners

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**Learning Overview:** After attending this presentation, attendees will understand the unique nature of stress in forensic science and how it may impact decision making. This presentation will also address the importance of workplace support and interactions and will present findings of a study conducted in forensic laboratories. In the study, data was collected on forensic examiners' experiences about workplace stress and support.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data and insights about an issue that has been largely overlooked in forensic laboratories: the experiences of stress and support and their impact on forensic decision-making. The data reveals the sources of workplace stress and levels of support experienced by forensic examiners, in addition to whether they consider stress as a factor that affects their judgments.

Forensic examiners operate in stressful environments where they are faced with various sources of workplace stress. Some stress factors, such as high caseloads and backlogs, are common across occupations. However, other factors, such as exposure to bloody crime scenes and working in a culture of "zero errors," are specific characteristic of forensic science. Constructive relationships and adequate support are primary factors associated with stress (or the lack thereof) among criminal justice employees in general. Forensic examiners interact and develop relationships with multiple stakeholders, some internal to their crime laboratory, such as managers and supervisors, and some external stakeholders, such as lawyers. These can be a source of stress, but can also be supportive and reduce stress.

One must also remember that stress is not necessarily negative. Stress, at moderate levels, can in fact be a motivating factor. Hence, undertaking research to understand and assess levels of support and the sources of workplace stress and their potential effects on forensic examiners' well-being and decision making is important, yet still lacking. While stress has been extensively studied for decades in many professional domains, forensic science has seen less than a handful of attempts to consider this human factor and its implication to forensic work.

The study reported here surveyed practicing forensic examiners ( $N=41$ ) from two laboratories. The forensic examiners were asked about the sources of their stress, its potential influence on their decisions, and the support they receive. Insights into the factors that may mediate stress were explored by investigating workplace stress as a function of the forensic field, the examiners' years of experience, and sex. Both descriptive and inferential statistics of the data are presented.

The data revealed that stress was caused by managers, supervisors, and case backlogs. Crime scene examiners felt higher stress than analytical examiners because of the nature of their cases and due to personal reasons. Female examiners felt more stressed than male examiners in general and also specifically in workplace stress. Different levels of experience affected two areas of stress—personal and work-related circumstances. No significant associations were found between management support and either general or workplace stress. Furthermore, the impact of stress on judgments provided a mixed finding; examiners were divided whether stress affected their judgments. The findings have practical implications for improving the decision-making environment of examiners. In the broader context, the development of a working culture that can address the negative impact of stress on examiners and its effect on forensic science judgements is needed.

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### Workplace Stress, Human Factors, Expert Decision-Making

## E79 Data-Driven Support for Optimal Forensic Laboratory Staffing

*Paul Speaker, PhD\*, West Virginia University, Morgantown, WV 26505*

**Learning Overview:** After attending this presentation, attendees will be able to effectively use an independent, data-driven tool to determine optimal forensic crime laboratory staffing (operational, support, and administrative personnel) and the corresponding necessary annual investment in equipment to sustain a given level of casework submission.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the laboratory-level response to the shortfall of forensic science positions at laboratories across the nation. Attendees will be able to immediately access the free, internet-based workforce calculator to determine the optimal staffing levels for their laboratory.

The December 2019 *Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices* Report to Congress from the Office of Justice Programs stated that publicly funded forensic crime laboratories require “... more than 900 additional full-time employees to efficiently address current caseloads.”<sup>1</sup> The 900-plus staffing gap represents state, metropolitan, county, and other regional laboratories across the United States (but does not address staffing needs at national laboratories). While the identification of the needs across the nation illuminates the depth of the shortage for Congress, it does not offer an indication of the specific staffing gaps at the local level. This macro level staffing shortage is translated to individual laboratory needs with a workforce calculator that was developed as a data-driven, independent assessment of laboratory staffing requirements. The use and interpretation of the workforce calculator will be demonstrated and explained in this session.

The workforce calculator is the product of a two-year timeline project commissioned by the Forensic Technology Center of Excellence. The calculator permits a laboratory to identify the personnel required to support a given level of casework within each area of investigation and the associated investment in capital equipment to support that level of activity. The first year produced an initial working tool (Beta Calculator), based upon a decade of performance of the most efficient laboratories as identified from Project FORESIGHT. In the second year, a more sustainable and more detailed econometric analysis as a long-term planning tool resulted, following practitioners reported experience with the Beta Calculator. These laboratories reported the Beta Calculator output along with their current workforce allocation across areas within the laboratory. In some cases, the laboratories indicated that the Beta Calculator output was consistent with their current allocation. In other cases, the Beta Calculator confirmed known or suspected understaffing in various areas of the laboratory.

This presentation includes a description of the underlying 12 years of Project FORESIGHT data, the efficiency criteria for laboratory data inclusion in the model’s data foundation, and brief descriptions of the econometric techniques deployed. The session emphasis is a demonstration of the MS<sup>®</sup> Excel<sup>®</sup>-based calculator, interpretation of the results, and the formulation of talking points for laboratory management to present to funding bodies. The underlying Project FORESIGHT data comes from the laboratories that qualified for the American Society of Crime Laboratory Directors (ASCLD) Maximus awards for laboratory efficiency. From the laboratories qualifying for 90% or better efficiency, 19 areas of investigation are examined for the efficient level of personnel output across caseloads, and an efficient frontier of analytical output is estimated. The demonstration includes detail on accessing the Forensic Technology Center of Excellence web-based calculator. Attendees are encouraged to bookmark the calculator site and evaluate current staffing and potential growth in caseload over time and corresponding growth in staffing needs to maintain efficient analysis of that caseload.

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1. National Institute of Justice. *Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices*. Report to Congress from the Office of Justice Programs. December 2019. <http://www.ncjrs.gov/App/publications/abstract.aspx?ID=279030>.

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### Workforce, Staffing, Management





## E80 The Implementation of a Blind Quality Control Program in a Forensic Laboratory

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**Learning Overview:** After attending this presentation, attendees will have learned how the Houston Forensic Science Center (HFSC) successfully implemented a blind Quality Control (QC) program, the benefits the program provides, the challenges faced when implementing and maintaining such a program, the results of the cases submitted, and the plans for the future of this program.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the difference between proficiency tests and blind QC tests and their benefits to a forensic laboratory. The intent of this presentation is to describe to attendees that a blind QC program in a forensic laboratory is possible as well as to encourage other laboratories to create a similar program of their own. Experiences, successes and failures, and tricks of the trade will be shared to benefit other laboratories and show that a blind QC program is an asset to laboratories individually as well as to forensic science as a whole.

As required by accreditation standards, forensic science service providers must participate in proficiency tests. These proficiency tests are often purchased from an external vendor and open in nature, which means the analyst is aware they are participating in a test. These tests often do not mimic normal casework in several ways, such as paperwork, packaging, and evidence type. While proficiency tests are beneficial for monitoring a laboratory's performance compared to other laboratories, they lack in providing insight into how the laboratory's internal processes and procedures are faring. Blind QC tests are intended to fill in the gaps that open proficiency tests leave behind. Blind QCs allow the laboratory to monitor the entire quality management system from submission of the evidence to the reporting of the results, and everything in between.

In 2015, HFSC adopted the recommendations by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) in the 2009 National Academy of Sciences (NAS) Report for blind proficiency testing by implementing a blind QC program.<sup>1</sup> The program is facilitated by the Quality Division, which is organizationally separate from the laboratory sections. Blind QC cases are prepared to appear like routine casework and submitted into the normal workflow. The cases are not expected to receive any special treatment by analysts, thus reducing the risk of intentional or unintentional bias in the analytical process. The Toxicology, Seized Drugs, Firearms, Latent Prints (Processing and Comparison), Forensic Biology, and Multimedia (Digital and Audio/Video) sections participate in the blind QC program.

Between September 2015 and December 2018, 973 blind QC cases were submitted throughout the six sections that participate in the blind program. Of those, 901 cases were completed and only 51 were discovered by the analyst as being a blind QC case. All 901 cases were completed satisfactorily. HFSC discovered two areas for improvement during this time frame due to the results of blind tests. As sample sizes increase, the goal of the program is to use the data generated to make error rate determinations. HFSC also intends to further collaborate with other laboratories in the forensic science community to expand on the opportunities that the program can provide by sharing knowledge, data, and resources.

### Reference(s):

- <sup>1.</sup> National Research Council (NRC) Committee on Identifying the Needs of the Forensic Science Community. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press; 2009.

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### Blind Testing, Proficiency Testing, Blind Quality Control



## **E81 Self-Inflicted Gunshot Wound: Suicide or Accident? You Decide**

*Erin P. Koester, MS\*, Department of Defense Office of Inspector General, Alexandria, VA 22350; Joshua Mayo, MSFS\*, Department of Defense Office of Inspector General, Alexandria, VA 22350; William B. Andrews, MFS, Alexandria, VA 22310*

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**Learning Overview:** After attending this presentation, attendees will understand the many challenges investigators face when evaluating crime scene evidence with a questionable manner of death.

**Impact on the Forensic Science Community:** This presentation will afford insight into the complexity of death investigations when there is a question of whether or not an individual self-inflicted a gunshot wound. The uncertainty of crime scene investigation and subsequent interpretation of an individual's actions should leave a lasting impact on attendees.

The discharge of a firearm, whether purposeful or accidental, leading to a self-inflicted gunshot wound, is a huge investigative challenge. Both purposeful and accidental discharges often contain similar evidence and case facts, and a decedent's sustained injuries, limited witness pool, and lack of circumstantial evidence (suicide note) only create more uncertainty.

When determining whether the manner of death is a suicide or accident, the forensic pathologist must evaluate whether an act caused an injury leading to death and whether the individual intended self-harm or death. For example, when a decedent dies during a game of "Russian Roulette," the forensic pathologist must determine whether the injury and subsequent death resulted from an intentional act or a mortal accident. Remember, high-risk behavior does not necessarily imply that a decedent intended death. While ultimately the forensic pathologist determines cause and manner of death, evidence an investigator uncovers greatly impacts that decision. The actions of the decedent immediately prior to the death, as well as, in the prior days, weeks, and months, can assist the investigators. Investigators may also use the decedent's social media activity, surveillance camera videos, and statements from friends, family, and others with whom the decedent may have had contact.

Two forensic science special agents will present a 2016 adult death case in which the forensic pathologist did not believe the manner of death was clear. They will provide information about the crime scene, evidence found, witness interviews, and other information to discuss the context around the cause of death. This presentation will explore the decedent's actions prior to the time of death and whether those actions, combined with the autopsy findings, resulted in an intentional self-inflicted injury causing death. They will present opposing interpretations of the evidence and argue those differing opinions. This presentation will conclude with the presenters challenging attendees to draw their own conclusions on the manner of death.

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### **Self-Inflicted, Suicide, Accident**

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## E82 A Case of Suicidal Hanging or Accidental Asphyxiophilia? A Case Discussion

*Sarah Hinnawi, MD\*, Mumbai, Maharashtra 400042, INDIA; Ganesh Patil, MBBS, HinduHrudaySamrat Balasaheb Thackarey Medical College, Mumbai, INDIA*

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**Learning Overview:** The goal of this presentation is to present a case of suicidal hanging that had some features of autoerotic death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into such cases that are brought for autopsy. Establishing the cause and manner of such cases is a challenging task. This presentation will provide important insight into the management of similar cases.

Suicide is the act of taking one's own life. It is often related to severe distress, the etiology of which is attributed to mostly psychosocial stressors and mental illness, such as depression, bipolar disorder, and schizophrenia, or alcohol or substance abuse. Autoerotic asphyxia is a paraphilia and category of sexual masochism characterized by self-strangulation up to the point of loss of consciousness to elicit or enhance sexual arousal. It is an accidental lethal sexual practice in which hypoxia is used to enhance orgasm by strangulation, hanging, or suffocation/exclusion of oxygen with a plastic bag. It is often accompanied with other paraphilias such as transvestism and bondage, a great range of paraphilias, sexual aids or pain-stimulating agents, pornographic magazines, intimate feminine garments, ropes, bondage, locks, chains, condoms, rubber items, and chemical anesthetics. The most characteristic feature is the protection of the neck by placing padding between the ligature and skin. Death is usually inadvertent. The practice usually involves young males in the second and fourth decade of life and is characterized by some activity to culminate in ejaculation. Frequently, it is also associated in transvestism. The activity is performed repeatedly, as shown in personal records, diaries, pictures, or local evidence such as grooves in woodwork or verbal communications from friends, partners, neighbors, and family concerning the nature of activities.

Death resulting from the practice of asphyxia, often associated with self-bondage for sexual purposes, creates a query in the mind of the examining forensic pathologist concerning the psychological impact and intentions of the deceased, as well as the manner of death. A case having both the characteristics of suicidal hanging and autoerotic death may lead to confusion in the mind of the forensic pathologist. It is especially important to distinguish from suicidal attempt, as it will be important in the diagnosis of future care or management in living persons. In the present case, the absence of certain characteristic findings as well as the history, the death was designated as accidental. The possibility of suicide must always be entertained, even in cases that have typical appearances.

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### Hanging, Autoerotic Death, Transvestism



**E83 The Impact of COVID-19 on Medicolegal Death Investigations: The Challenges, Strategies, and Lived Experiences During the Early Days of the Coronavirus Pandemic**

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**WITHDRAWN**

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## E84 Managing COVID-19-Related Death in Ibadan: The Human Angle Narratives of a Forensic Pathologist

*Uwom O. Eze, MD\*, University College Hospital (UCH), Ibadan, Oyo State 23402, NIGERIA*

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**Learning Overview:** After attending this presentation, attendees will reflect more deeply on background factors that may have a considerable influence on procedures and outcomes when determining manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that not all forensic-related issues are technical and sometimes the human angle story may be insightful.

The occasional outbreak of diseases in Africa, including the recent Ebola infection in parts of the region, is not something new. However, nothing prepared Africa for the COVID-19 pandemic. Regrettably, the initial local responses to the pandemic did not have any significant operational component for management of the dead following COVID-19. The approach to any resulting mortality was essentially reactive when the first suspected COVID-19 death occurred, thus laying bare the unpreparedness and attendant conundrum. Complicating the situation is the fear of the novel virus among health care workers, including medical doctors, which was clearly expressed and the manifestations were palpable. It significantly impacted the attitude and manner of response to patients presenting with any illnesses who may have had any symptoms that could be readily or remotely linked with COVID-19. Ironically, COVID-19 “suspected” status is direr than being a confirmed case. The “suspected” patients are often left in a dangerous gray zone, where, with limited Personal Protective Equipment (PPE) and restricted access to testing and available resources prioritized for the care of diagnosed cases, the outcome is not uncommonly deadly. Coupled with no facilities for safe autopsy and a lack of body-holding areas for either suspected or confirmed cases of COVID-19, determining the cause and manner of death and dealing with the anguish of families of the deceased persons present one of the toughest challenges. There are frequent complaints from family members of the deceased about the neglect of their loved ones at health facilities, and they often blame it for being responsible for the death of the patient. The whole cloud of uncertainty, mixed messages, including “facts” about COVID-19 that change over time and “myths” that evolve, and confusion about what to believe or disregard, complicate the picture. Some notion that the pandemic is either a hoax or some conspiracy is gaining ground, and this disposition confounds the efforts of an appropriate response, including managing bodies of the dead, where the cooperation of bereaved families is vital.

How does one objectively evaluate the impact that fear and attendant reluctance by health care workers to provide care to patients with COVID-19-like symptoms have on the death of such patients? How does one deal with a situation where, after a family had requested a copy of a COVID test result of their deceased loved one, tore it up, claiming it was all contrived and a false report? How do professionals begin to investigate the death when there is no appetite whatsoever by the relevant agencies to be involved in any form of contact for such a purpose, fearing their health and safety? How does one begin to engage the public and create awareness on issues about the management of COVID-19-related deaths and their implications when public officials and health managers do not want to reckon with COVID-19 mortality because it is “sensitive” and goes against official narratives about a “successful response”? How does one achieve dignified handling of bodies of the dead from COVID-19, including respect for their families and safe burial within a short time, considering peculiar circumstances? These conditions include limited resources and a socio-cultural milieu that place a high premium on specific final rite rituals, which are incompatible with infection prevention and control measures required for COVID-19. The practical reality and science of COVID-19-related deaths in this context are incomplete without human angle narratives.

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### Management of the Dead, COVID-19, Human Angle

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## E85 A Medicolegal Death Investigation and Review of Inhalant-Related Deaths

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**Learning Overview:** After attending this presentation, attendees will understand a step-wise approach for the medicolegal death investigation of suspected inhalant-related deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of a thorough medicolegal death investigation of suspected inhalant-related deaths because forensic pathologists heavily rely on the investigative findings when opining cause and manner of death in these cases.

Inhalation-related deaths of gases and volatile products like hydrocarbons found in compressed air duster cans represent a small subset of drug-related deaths investigated by medical examiner offices. The medicolegal death investigation is critical in identifying possible inhalation-related deaths because many of these gases and volatile products are either not detectable on postmortem toxicology testing or are not included on routine postmortem toxicology testing. Therefore, the cause and manner of death in these cases heavily rely on a thorough medicolegal death investigation.

A review of the medical examiner database of deaths in western Michigan was completed over an 11-year time period, from 2009 to 2020. During this period, there were 26 inhalation-related deaths. The average age of inhalant-related deaths was 44 years (range: 21 years to 76 years), there were 17 males (65%) and 9 females (35%), and 25 (96%) were White and 1 (4%) was Black. The location of inhalant-related deaths was predominantly in the decedent's residence (17 cases, 65%), followed by far less-common locations, which included the hospital (2 cases, 8%), outside (2 cases, 8%), motor vehicle (1 case, 4%), camper (1 case, 4%), and motel (1 case, 4%). Two cases had limited information in the database, which did not include the location of death. Inhalants identified on toxicology testing or based on detailed review of the medicolegal death investigation and listed as the cause or contributing to the cause of death included: helium (9 cases, 35%); 1-1, difluoroethane (7 cases, 27%); nitrogen gas and nitrous oxide (2 cases, each); and dimethyl ether, chloroform, methane, butane, and hydrofluorocarbon not otherwise specified (1 case, each). The manner of death was opined suicide in 13 cases (50%), accident in 12 cases (46%), and undetermined in 1 case (4%). All of the helium deaths were opined suicide and all of the 1-1, difluoroethane deaths were opined accident.

Given the heavy reliance on a complete medicolegal death investigation in suspected inhalant-related deaths, this presentation proposes a step-wise approach for medicolegal death investigators to ensure forensic pathologists are best positioned to opine the cause and manner of death in such cases: obtaining a detailed history of medical conditions and social activities, including psychiatric history, risk-taking behaviors, and recent reported mental state and behavior of the decedent just prior to being found dead; speaking with the individual(s) who found the decedent to get the most accurate description of the undisturbed death scene; taking several photographs of the decedent with a focus on areas near the mouth and nose for residue, and also photographs of the area around the decedent, to include trash cans, closets, cloths, rags, plastic or paper bags, and adjacent rooms, if applicable; if identified, taking multiple photographs of the suspected gas or volatile product container so the forensic pathologist and toxicologist have an idea of what the decedent may have inhaled; and, if deemed necessary, saving the suspected gas or volatile product container for direct toxicology testing.

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### Inhalant, Investigation, Forensic

## E86 A Postmortem Medicolegal, Radiological, and Toxicological Investigation in a Case of Suicide by Multiple Stabbings Associated With Ingestion of an N-Hexane Mixture

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of a multidisciplinary investigation in a case of suicide by multiple stabbings and acute intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of postmortem medicolegal, toxicological, and radiological investigation by Postmortem Computed Tomography (PMCT) and Postmortem Cardiac Magnetic Resonance (PMCMR) in a case of suicide.

N-Hexane is a colorless liquid with a gasoline-like odor that is mainly used, mixed with other substances, as a solvent in cleaning agents, in the printing, textile, furniture, and shoemaking industries, and in the manufacture of pharmaceuticals.<sup>1</sup> Exposure to n-hexane most frequently occurs among industrial workers in occupational settings. Nevertheless, due to its easy accessibility, solvents and glues containing n-hexane are often used as inhalant abuse by “sniffers.”<sup>2,3</sup> Human data on the acute toxicity of n-hexane are extremely limited. The acute toxicity of n-hexane is very low, and no cases of lethality were reported after inhalation of n-hexane or its mixtures. According to research, there are no published cases of n-hexane ingestion, apart from a study conducted on rats that excluded liver toxicity after oral intake.<sup>4</sup>

The evaluation of stab wounds, well-known in the forensic field, has been recently implemented by postmortem radiological studies for the evaluation of the injury path. Only a few published cases studied the wound trajectory by performing postmortem imaging with the weapon still embedded.<sup>5,6</sup> Presented here is a toxicological and radiological evaluation in a case of suicide by multiple stabbing associated with the ingestion of an n-hexane mixture.

A 41-year-old man was found in his car, sitting on the driver’s side with a kitchen knife embedded in his chest. Other stabs were found in the thoracic and abdominal area. The man held in his right hand an empty bottle of a cleaning product containing n-hexane. Before performing the autopsy, a total body Postmortem Computed Tomography (PMCT) was performed in order to study the pattern of injuries. The radiological examination, conducted with the knife still embedded in the chest, revealed that there were several cutaneous and subcutaneous lesions of the chest, contiguous to the knife, massive bilateral hemothorax, left pneumothorax, and massive hemopericardium. Apart from the stab wound with the knife still embedded, the external examination showed that there were 14 other stab wounds, mostly in the precordial area, and the remnants in the abdominal area. All the lesions had an ogive shape, with the acute angle on the right side, and all had similar measures. The autopsy was conducted using a layer-by-layer technique, revealing that the majority of the lesions were very superficial, as they injured only the muscles. The deeper stabs were one in the abdominal area, that had superficially injured the right part of the liver, and the one with the knife still embedded. The tip of the blade protruded from the sternum for 32mm. The autopsy confirmed the presence of massive bilateral hemothorax and pericardium lacerations. The heart revealed a stab wound of the anterior wall, proximal to the apex. The heart was fixed in formalin, and a Postmortem Cardiac Magnetic Resonance (PMCRM) was performed, confirming the presence of a full thickness, incised wound penetrating the anterior wall of the heart, near the apex. The histological examination confirmed the vitality of all the lesions. The toxicological examination revealed the presence of a mixture of hexane isomer in the blood and in the stomach, compatible with the cleaning product found in the car. It confirmed that acute intoxication occurred, though no lethal effects could be attributed to this substance.

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### N-Hexane Intoxication, PMCT, PMCMR

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## E87 A Longitudinal Evaluation of Death Investigation of Elder Abuse and Neglect Deaths

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**Learning Overview:** After attending this presentation, attendees will be able to apply initial screening criteria in elderly deaths to identify when additional death investigation is necessary to rule out abuse and neglect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in two ways. First, it establishes a prevalence of deaths with first-level suspicion of elder abuse and neglect in a large metropolitan area; secondly, it provides criteria to quickly screen elder deaths for abuse and neglect in order to identify cases that need further investigation.

The purpose of this study was to develop simple criteria to screen elder deaths to ascertain which deaths require more intensive investigation in order to rule out abuse or neglect. This study retrospectively applied these criteria to a five-year longitudinal population of deaths undergoing a full autopsy at a large metropolitan medical examiner office. The deaths identified with these screening criteria (hereafter referred to as “first-level suspicion of abuse”) comprise approximately 10% of deaths  $\geq 65$  years old.

This was a retrospective study (2015–2019). The population included all deaths  $\geq 65$  years old, and the researchers included only cases where the body was brought to the office for further examination (autopsy or external). However, only deaths undergoing a complete autopsy were screened. The criteria for first-level suspicion included: (1) dependence on another person(s) for at least one Activity of Daily Living (ADL), and (2) at least one marker/indicator of abuse or neglect. The markers included seven items: delay in seeking care for an injury or acute change in health status; suspicions expressed by health care workers, law enforcement, family or friends; unclean or unlivable living space; no physician visit (or ongoing prescriptions) within one year of death WITH documented medical history; poor hygiene; unexplained physical trauma; and suspicious circumstances. This study also established criteria for exclusion; some of these were obvious (e.g., death in a motor vehicle crash), while some were more subjective (hoarder residence, homeless, substance abuse).

Of the 5,298 decedents  $\geq 65$  years old who were examined, 2,798 (53%) received complete autopsies and 2,500 (47%) external examinations. Of the 2,798 receiving an autopsy, 474 (17%) screened positive for first-level of suspicion of abuse. These cases had several identified areas of dependence on another for an ADL of which 220 (46%) mobility/transferring, 154 (32%) maintaining continence, 58 (12%) dressing, 53 (11%) eating and met at least one marker/indicator of abuse or neglect. Specifically, 322 (70%) had injuries, 180 (38%) had a reported suspicion of abuse or neglect surrounding the death, 107 (23%) had a change in health or injury with a delay in seeking care, 77 (16%) had not seen a Primary Care Physician (PCP) within a year with documented medical history, 71 (15%) had the presence of and/or report of poor hygiene, 34 (7%) had a history of interpersonal violence, and 32 (7%) resided within an unclean/unlivable living environment.

The total number of male and female decedents were 238 (50%) and 236 (50%), respectively. Race/ethnicity included non-Hispanic White 270 (57%), Black 118 (25%), Hispanic 67 (14%), and other 19 (4%); the latter includes Asians, American Indians, multi-ethnic, and unknown. The age median (Interquartile Range [IQR]) was 77.05 (69.6–86.2). Of the 474 deaths, 326 (69%) had been residing at a personal residence at the time of death, and 148 (31%) had resided within an environment with third-party providers (nursing home, assisted living, group home). Moreover, 174 (37%) had a recognized cognitive impairment, 109 (23%) had decubitus ulcers with 29 (27%) receiving care. The manner of death breakdown was as follows: 264 (56%) natural, 144 (30%) accident, 44 (9%) homicide, and 22 (5%) undetermined. Lastly, 236 (49.8%) of the deaths had a scene investigation with the remainder dying within an in-hospital setting.

These criteria can be quickly applied in prospective death investigations as they are reported in order to identify deaths that require further investigation for abuse and neglect. The prevalence of these deaths, even in a large medicolegal jurisdiction, is relatively low, despite the large number of deaths in the  $\geq 65$  years old age category. It follows that more intensive investigation of deaths after application of these criteria is realistic, even in this large jurisdiction.

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### Forensic Science, Elder Abuse and Neglect, Elderly Death Investigation





## **E88 A Unique Case of Postmortem Manipulation Artifact Mimicking Homicidal Violence**

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**WITHDRAWN**

## E89 Deceiving Appearance: Death Due to the Use of the Novel Psychoactive Substance Bromazepam

Anita Roman Hasert, BS\*, Charleston County Coroner's Office, North Charleston, SC 29405

**Learning Overview:** After attending this presentation, attendees will be able to apply their understanding of thorough medicolegal death investigation practices toward scene investigations and the importance of communication. Attendees will learn the importance of a multidisciplinary team approach in novel psychoactive substance-related death investigations. Attendees will learn the significance of effective communication and collaboration with law enforcement, medical professionals, and forensic specialists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to both the literature regarding bromazepam intoxication and why a multidisciplinary team approach is vital for complete investigations. Medicolegal death investigation policies and procedures need to ensure accurate scene investigation to include: collection of substances on scene, both known and unknown, and ensure that testing analysis is completed on those substances that were collected in the field. This presentation will also provide information on clandestine drug labs, to include the pill printing process.

The drug class benzodiazepines works to calm or sedate a person by raising the level of the inhibitory neurotransmitter Gamma-Aminobutyric Acid (GABA) in the brain and was first identified back in 1955, by chemist Leo Sternbach. It would later be the most prescribed medication class in the world.<sup>1</sup> According to literature, bromazepam was first patented in 1963 and in the 1970s, began being clinically used. Bromazepam is the common name; there is a full list of brand names from countries all over the world.<sup>2</sup> Bromazepam has Central Nervous System (CNS) depressant properties and shares anticonvulsant, muscle relaxant, hypnotic, anxiolytic, and sedative effects like other benzodiazepines.<sup>3</sup> Currently, bromazepam is not prescribed in the United States for various possible reasons; it could be because it has not been approved by the Food and Drug Administration or because of its high likelihood of abuse and addiction.<sup>4</sup>

The decedent was a 23-year-old Caucasian male who was found unresponsive by his college roommate on the floor of his bedroom. He had struggled with illicit drug use and prescription drug abuse for the past few years and had refused to seek any rehabilitation centers. He had moved to Charleston, SC, about two years ago to attend college and the last time the family had spoken to him, he reported that he was going to quit using drugs and was excited for school to start. On the scene investigation, drug paraphernalia was found to include a syringe, spoon with residue, a plastic bag with tan powder, white rectangular pills, and loose round gray/white pills. The drug paraphernalia was collected, minus the round gray pills. Prolonged use of bromazepam can result in dependency of the drug due to a buildup of tolerance and can also cause problems with brain and body functions such as learning ability, memory, and sensory perception. Bromazepam is long-acting and is metabolized through the liver via oxidative pathways. The appearance of the drug in pill form is as a tablet, but it is a white, odorless powder. The round gray/white pills that were loose in the decedent's dresser had a slight imprint on them and the color just looked like gray and white powder pressed into a round shape but, despite trying to determine what the pills were, they were never collected. Upon returning to the residence the following day, the pills were already missing. Although the drugs that were tested did not return as bromazepam, alprazolam and heroin were detected in the other collected paraphernalia. In conducting research, there are multiple articles regarding pharmacokinetic parameters of bromazepam. Based on the circumstances surrounding the victim's social history, toxicology, drug analysis, and autopsy findings, the cause of death was determined to be acute mixed drug toxicity, with bromazepam as one of the drugs attributing to the death. The manner of death was classified as accident.

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### Benzodiazepine, Bromazepam, Death



## F1 Lessons Learned From the Creation and Operation of Conviction Integrity Units (CIUs) in District Attorney's Offices in Texas

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**Learning Overview:** The goal of this presentation is to help attendees understand the intersection of forensic science and legal issues in the context of actual innocence case reviews in CIUs in Texas. Attendees will learn about the impact of advances in forensic sciences on the viability of actual innocence claims and on the role of forensic scientists in advancing the interest of justice in these cases. Attendees will also better understand the issues facing non-forensic science practitioners as they seek to understand emerging issues in forensic sciences and their impact on evidence in actual criminal cases. Finally, attendees will have an appreciation for the collaborative approach to conviction integrity that differs from the traditional adversarial court system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic scientists and legal practitioners see the role of forensic science in the context of the legal standards and procedures required to establish a successful claim of actual innocence. This presentation will also help forensic scientists understand how to better communicate changes in forensic sciences to non-scientist consumers.

**Synopsis:** According to the National Registry of Exonerations, there have been over 2,600 exonerations in the United States since 1989. Of the six CIUs currently in operation in Texas, the oldest was established in 2007, while the newest was started in 2019. These units often must navigate the intersection of forensic science, rules of evidence, substantive post-conviction legal standards, and the interests of justice. The prosecutors assigned to these units each bring different levels of experience and different areas of expertise to the role. Texas statutes mandate that the duty of a prosecutor is "not to convict, but to see that justice is done." However, the day-to-day practice in a prosecution office can lead to a mindset that places confidence in the "rightness" of a conviction and that views actual innocence claims as inherently suspect. One of the first things a prosecutor assigned to a CIU must do is adjust their mindset and accept the possibility that the criminal justice system sometimes makes mistakes.

Forensic evidence, particularly DNA evidence, has played a pivotal role in many exonerations. In some cases, advances in the analysis of forensic evidence have allowed for previously untested evidence to be used to free someone who was wrongfully convicted. Advances have also resulted in the re-evaluation of previously tested evidence that impacts the role that evidence played at trial. Prosecutors assigned to a CIU may have limited experience in forensic sciences. One of the challenges they face is learning to communicate effectively with forensic scientists in order to understand and assess the nature of the forensic evidence presented.

Forensic scientists may be called upon to re-examine prior work considering advances in the underlying science or its application to casework. The requests can come from the state or from the defense. This suggests a need for policies for addressing these requests. Forensic scientists may have a duty to review past work when changes in the field call into question prior cases or testimony and to notify prosecutors or defense attorneys if the results change significantly.

In some cases, the new forensic evidence clearly establishes the convicted person's innocence. However, in many cases, the forensic evidence must be considered in the context of other evidence. Prosecutors must rely on their practical experience in assessing the evidentiary impact of changed forensic evidence. One of the greatest challenges facing a prosecutor in a CIU is how to address a case where new forensic evidence causes one to seriously question a conviction but does not go so far as to conclusively establish actual innocence.

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### Conviction Integrity, Exoneration, Actual Innocence



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## F2 Licensing of Forensic Analysts in Texas and Implications for Individual Professional Misconduct

*Leigh M. Tomlin, JD\*, Austin, TX 78701*

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**Learning Overview:** After attending this presentation, attendees will understand the oversight approach taken in Texas with respect to individual forensic analysts, including licensure of practicing forensic analysts and disciplinary action taken pursuant to a finding by the Forensic Science Commission (FSC) of professional negligence or misconduct.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating one approach to tracking negligence and misconduct by state forensic practitioners, including examples of disciplinary action taken by the Commission pursuant to its investigative and forensic analyst licensing authority.

In Texas, the FSC is the oversight body tasked with improving the integrity and reliability of forensic science in Texas criminal cases. As part of its mandate, the FSC investigates allegations of professional negligence and misconduct in accredited crime laboratories—allegations that are received in the form of a complaint or a required disclosure from a crime laboratory. In addition to its investigative authority, the Texas Legislature more recently tasked the Commission with creating a forensic analyst licensing program. Forensic analysts in Texas were required to be licensed by the Commission effective January 1, 2019. As part of its licensing authority, the Commission may take disciplinary action against a license holder or applicant for a license after an investigative finding of professional misconduct or on a determination a license holder has violated a rule or order of the Commission, including violations of the Texas Code of Professional Responsibility for Forensic Analysts and Crime Laboratory Management. Findings by the Commission with respect to analyst conduct are based on the outcome of a full investigation by the Commission and described in a publicly available investigative report.

Disciplinary action may include revocation or suspension of a person's license, refusal to renew a person's license, reprimanding the license holder, or denying a person a license. The Commission may also place on probation a person whose license is suspended. Where a license suspension is probated, the Commission may require the license holder to report regularly to the Commission on matters that are the basis of the probation or continue or review continuing professional education until the license holder attains a degree of skill satisfactory to the Commission in those areas that are the basis of the probation. In instances where the person's license is suspended or revoked, the Commission records and publishes this information on its website.

Recently, the Commission revoked a Seized Drugs Analyst's license as a result of its investigation of a self-disclosure by the employing laboratory in which the analyst engaged in the inappropriate manipulation of data during a competency test for a new qualitative analytical method. The method was used to distinguish legal hemp from illegal marijuana. The analyst acknowledged having manipulated the data when confronted. In its normal course of an investigation, the Commission notified the analyst of its investigation of the self-disclosure but never received a response from the analyst. Based on the information received from interviews with laboratory staff and documentation provided by the laboratory, the Commission found the act of data manipulation by the analyst constituted professional misconduct and recommended revocation of the analyst's license. The Commission notified the analyst of the finding and her right to appeal the finding, but received no response. Subsequently, the analyst's license was revoked, and the revocation was recorded in the Commission's published list of licensees.

In its consideration of disciplinary actions to be taken against an analyst, the Commission carefully considers mitigating and aggravating factors and circumstances specific to the incident, including an analyst's willingness to come forward and own up to a mistake or an analyst's ability to be retrained or rehabilitated.

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### Forensic Analyst Licensing, Oversight Regulation, Misconduct



## F3 Forensic Laboratories: Time to Lawyer Up

*Amy Curtis Jenkins, JD\*, Virginia Department of Forensic Science, Richmond, VA 23219-1416*

**Learning Overview:** The goal of this presentation is to detail the role of in-house counsel in the forensic laboratory setting and describe the guidance that an in-house attorney can provide to forensic scientists and laboratory management, as well as the advocacy needed for the forensic laboratory in the criminal justice system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need to have an attorney advising the laboratory who is familiar with the forensic science disciplines and bound by the same ethical guidelines as the forensic scientists, as well as knowledgeable of how criminal cases are handled in the system.

The in-house counsel for the laboratory has a number of duties, including (but by no means limited to): (1) responding to requests for case documentation and other records, (2) overseeing the laboratory's compliance with court-ordered pre-trial and post-conviction testing orders, (3) managing ongoing case reviews conducted by the laboratory, (4) providing advice on human resource issues and providing required *Giglio* disclosures, (5) advising laboratory management on compliance with federal and state laws and regulations, (6) advocating for the laboratory in the legislative process and in the development of legal rules and procedures, (7) providing legal training for the forensic scientists, and (8) conducting outreach to the legal community.<sup>1</sup>

An in-house counsel will learn that scientists and lawyers do not always communicate well with each other. Legal requirements like *Giglio* disclosures are not well understood by forensic scientists, and most criminal lawyers lack any significant understanding of the scientific principles and processes involved at the lab.<sup>1</sup> In-house counsel can help bridge the gap by encouraging/facilitating communication and providing information.

The 2009 NAS Report, *Strengthening Forensic Science in the United States: A Path Forward*, recommended that funding be provided so that state and local jurisdictions could remove "all public forensic laboratories and facilities from the administrative control of law enforcement agencies or prosecutors' offices."<sup>2</sup> Independence of a forensic laboratory is dampened if the legal advisors provided for that laboratory are employed in prosecutors' offices whose concerns regarding the finality of judgments and political pressures may run counter to the laboratory's ethical obligations to correct errors and honestly communicate with all parties. An in-house attorney, reporting only to laboratory management, can be a more effective advocate for a neutral, transparent laboratory. An in-house attorney who also understands the potential impact of forensic errors on the criminal justice system can better inform and advise laboratory management.

In-house attorneys also need an opportunity to discuss current issues and learn from each other's experiences (and failures) as they navigate their roles. This study has been working with other laboratory counsel to arrange national trainings and develop an association to facilitate ongoing communication and trainings for this previously-siloed group. Advancement of forensic science requires that forensic laboratory counsel work to develop consistent policies and procedures for a level playing field for all participants.

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### Laboratory Counsel, In-House Counsel, Laboratory Lawyer



## F4 Implementing Organization of Scientific Area Committees (OSAC) Standards at the Local Level: Lessons From Texas

*Lynn Garcia, JD\**, Texas Forensic Science Commission, Austin, TX 78701; *Mark D. Stolorow, MS, MBA\**, National Institute of Standards and Technology Special Programs Office, Gaithersburg, MD 20899-8602

**Learning Overview:** After attending this presentation, attendees will have learned about efforts in Texas to implement OSAC standards in a variety of laboratory settings as well as the development of standards-related educational tools for lawyers and judges.

**Impact on the Forensic Science Community:** As OSAC nears the end of its sixth year of operations, we are seeing the fruits of several years and thousands of hours of labor. This presentation will impact the forensic science community by discussing successes and challenges in making the standards and guidelines work for laboratories with varying resource levels. The critical task of educating lawyers and judges on the significance and scope of OSAC work product will also be discussed.

Since its official inception in 2014, the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees for Forensic Science (OSAC) has worked to advance the mission of strengthening forensic practice by facilitating the development of science-based standards through a formal Standard Developing Organization (SDO) process, evaluating existing standards published by SDOs for placement onto the OSAC Registry, and promoting the use of the OSAC Registry-approved standards throughout the forensic science community.

While this effort is widely embraced by forensic science service providers and stakeholders of the criminal justice system in theory, the “how to” of practical implementation is far more challenging. This is especially true considering accrediting bodies such as the American National Standards Institute (ANSI) National Accreditation Board (ANAB) and American Association for Laboratory Accreditation (A2LA) will not assess laboratories to OSAC standards unless and until the laboratories adopt those standards as part of their operating procedures.

Further complicating the effort is the fact that most criminal cases are prosecuted in state court. There is no national agency or regulatory body to act as the “owner” of the forensic science enterprise. This leaves state oversight bodies like the Texas Forensic Science Commission in the unique and enviable position of benefitting from the work product emerging from the OSAC and determining when and how specific expectations regarding the adoption of OSAC Registry documents should be established. In Texas, the Commission’s decisions with respect to OSAC implementation impact just under 100 laboratories, approximately half of which are physically located inside Texas and the other half physically located outside of Texas.

As OSAC nears the end of its sixth year of operations, we are seeing the fruits of several years and thousands of hours of labor. By September 30, 2020, there were 37 standards on the OSAC Registry of Approved Standards and, according to the most recent OSAC Standards Bulletin (September 2020), there are several hundred standards at some point in the OSAC or related SDO process.

With the list of OSAC Registry standards growing exponentially, the Texas Commission has begun a process of reviewing standards and guidelines and making recommendations with respect to laboratory adoption. The Commission’s initial focus is on encouraging a period of voluntary adoption before any mandates take effect. In encouraging voluntary adoption, the Commission recognizes not all laboratories have the same resources, and not all standards and guidelines are created equal. While some are obvious for adoption, others may be perceived by local forensic science service providers as going either “too far” or “not far enough,” depending upon the reader’s perspective.

What is critically important, however, is that the Texas Commission embraces the OSAC process overall while simultaneously listening to the needs of Texas laboratories and providing implementation resources to the extent possible. These resources are in the form of discipline-based committees that will review and provide observations to the Commission, assistance with gap analyses to assess where labs stand relative to published standards, and training and educational resources from members of the OSAC most closely involved in developing the standards. For some standards, it may also be the case that the Commission adds supplementary comments with respect to certain aspects of the documents that could be served by additional clarifying information the Commission believes important to ensuring appropriate adoption of sound scientific principles.

In addition to the state-based standards vetting process, the Commission is in the process of publishing the OSAC standard-adoption status of each publicly funded crime laboratory within its jurisdiction, as well as any privately funded laboratories (e.g., Bode, NMS Labs, Signature Science, and others) that perform casework in Texas and choose to be included in the list. This information provides the criminal justice community with an understanding of what to expect from laboratories in various disciplines. Finally, the Commission will offer virtual training for the Texas legal community on every standard reviewed and recommended for adoption. This will assist the ultimate end-user (the criminal justice system) with understanding the purpose and scope of each document.

Finally, the OSAC Quality Task Group has created a “How-to Guide” to help quality managers, senior management, and technical leaders with step-by-step tips for implementing standards, including language to add to methods manuals and quality assurance manuals. The [“How-to Guide” for standards implementation](#) is posted on the [OSAC website](#).

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### OSAC, Texas, Implementation



## F5 Putting Words in the Mouth of the Expert: Using Rules of Evidence to Script Expert Testimony Based on the President’s Council of Advisors on Science and Technology (PCAST) Principles

*Michael Chamberlain, JD\*, California Department of Justice, San Francisco, CA 94102*

**Learning Overview:** The goal of this presentation is to inform and inspire discussion about efforts to implement forensic science “reform” by amending rules of evidence to require judges to dictate the wording that may be used by experts to express their opinions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware of the effect rules of evidence can have on how expert opinions are structured and phrased. This presentation will also ensure that the community is aware of the continuing influence the PCAST Report is exerting in a context that is, perhaps, unexpected.<sup>1</sup>

What if there were a rule of evidence that required judges to instruct expert witnesses what words they could and could not use in conveying their opinions to the jury? And what if the guidance provided to judges on how to dictate expert witness rhetoric was informed by principles set forth in the 2016 PCAST report?<sup>1</sup> Who, exactly, would be testifying? The expert? The judge? The PCAST authors? These are questions that may well become relevant in the federal trial courts.

The past decade has seen a healthy and productive discussion about how to enhance the reliability of forensic science evidence received in criminal trials. Some prominent products of that discussion have been national publications such as the 2009 National Academy of Sciences (NAS) Report on forensic science, the National Commission on Forensic Science, the 2016 PCAST Report, and the creation of the OSACs, not to mention scores of AAFS talks.<sup>1,2</sup> There has been a simultaneous push by some for a mechanism by which to “regulate” forensic science according to the principles articulated in national publications such as PCAST. This has proved difficult to implement. So, a new tactic is being pursued: turn trial court judges into regulators by amending rules of evidence to require that judges shape expert testimony consistent with PCAST principles.

As of late 2020, the federal Advisory Committee on Rules of Evidence continues to consider amending Federal Rule of Evidence 702, which governs the admissibility of expert witness testimony. The stated objective of the amendment is to prohibit “overstatement” by experts when providing their opinions and conclusions. The means by which this would be accomplished is by providing judges with a “note” about how to implement the amended rule. This proposed note, in its current draft iteration, would be informed and animated by the views expressed in the PCAST Report.<sup>1</sup>

This presentation will discuss efforts at both the state and national levels to affect forensic science reform through rules of evidence. It will pose questions about the merits and drawbacks to using rules of evidence in that manner. For example, established procedures typically require judges to screen proffered scientific evidence by assessing the fundamental validity of the methods relied upon and whether the resulting opinion is logically derived from those methods. Could a rule requiring more myopic focus on the wording of the opinion deprive the jury of evidence that may otherwise survive that screening process? And, would restricting an expert’s language choices be equally applicable to all forensic science disciplines in all factual settings, or would a “one-size-fits-all” rule unnecessarily restrict judicial discretion to issue rulings in the context of unique case facts?

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### PCAST, Rules of Evidence, Experts

## F6 Science, Technology, and Jurors: An Update

Donald E. Shelton, JD, PhD\*, University of Michigan-Dearborn, Dearborn, MI 48128-2406

**Learning Overview:** After attending this presentation, attendees will be aware of the potential impact on jurors of the scientific and technological developments of the past decade, particularly the “Tech Effect” and the “CSI Effect” myths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware of how the scientific and technological developments of the past decade may impact the attitudes of jurors toward forensic science evidence in criminal cases.

Ten years ago, the validity of the so-called “CSI Effect,” which alleged that jurors were wrongfully acquitting criminal defendants when the prosecution did not present the type of forensic science evidence seen on television programs like *CSI* was examined. While data did demonstrate that jurors expected, and in some cases demanded, scientific evidence, there was no correlation with the television programs those jurors watched.<sup>1-4</sup> It is suggested that those juror expectations and demands were more likely partially the result of what was called a “Tech Effect”—the impact of the technological and informational revolution in our society—which informed jurors about the ready availability of DNA and other forensic science techniques.

It is time to take another look. This presentation discusses the impact of some of the technical and social changes in the past decade on today’s jurors and discusses whether the “Tech Effect” persists considering those developments.

Some of those developments include increased public awareness of the large numbers of exonerations. The National Registry of Exonerations documented 2,665 exonerations of wrongfully convicted persons since 1989. Almost 1,300 of those exonerations have come since 2010. It also includes new developments in DNA techniques, including such things as autosomal Short Tandem Repeats (STRs), Y-chromosomal Short Tandem Repeat s (Y-STRs), Single Nucleotide Polymorphisms (SNPs), body fluid identification, rapid DNA testing, Next Generation Sequencing (NGS), DNA mixture interpretation, Probabilistic Genotyping Software (PGS), DNA phenotyping, and new genomic platforms regarding “touch” and other refinements. Another development is the use of genetic genealogy to solve crimes and the widespread publicity involving its solving of “cold” cases.

Meanwhile, there also is a growing politically based “anti-science” movement. There has been a demonstrated policy rejection of previous forensic science efforts. Recently, there has been a very public debate about the seemingly “anti-science” approach to the COVID-19 pandemic in addition to media coverage of forensic science laboratory misconduct. There have been several highly publicized cases of forensic laboratory fraud and mistakes and, relatedly, a distrust of police testimony and conduct following video documentation of police misuse of force. The Black Lives Movement is just the latest result of such technologically documented police misconduct.<sup>5-12</sup>

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### Jurors and Science, CSI Effect, Tech Effect



## F7 Transfer and Persistence Expert Evidence in the Canadian Criminal Trial Process

*Cecilia Hageman, PhD\*, University of Ontario Institute of Technology, Oshawa, ON L1H 7K4, CANADA*

**Learning Overview:** After attending this presentation, attendees will better understand how Gun Shot Residue (GSR) evidence and DNA transfer and persistence evidence have been interpreted and used by non-scientist justice system participants in the Canadian criminal trial process.

**Impact on the Forensic Science Community:** Canadian criminal trials are neither videotaped nor televised, and expert forensic witnesses are generally not present during times when their evidence is summarized and argued, such as in opening and closing statements by lawyers or in judges' instructions to the jury or rulings from the bench. Experts, therefore, are generally unable to determine how their evidence is actually used in the trial proceeding in real time. This presentation will impact the forensic science community by relating the purpose of this research, which was to determine whether in-trial statements from lawyers and judges regarding the expert testimony in DNA or GSR transfer and persistence, made during the absence of the expert, were accurate.

Relevant cases were targeted by searching selected keywords in the Lexus Advance Quicklaw and the Canadian Legal Information Institute (CanLII) public databases. Trial documents were obtained from the Ontario Court of Appeal Records Office in Toronto, Canada, where files already contain full transcripts for criminal appeal purposes. Qualitative coding of transcript testimony was performed by locating those transcript statements relating to the transfer or persistence of DNA or GSR, comparing those statements to the experts' statements, then assessing and categorizing the statements as accurate or not. In particular, transposed conditional statements were highlighted, as were instances of lawyers proffering transfer and persistence inferences without expert input.

While too few cases (ten) were analyzed for statistical purposes, general trends were noted. For the majority of the GSR cases, lawyers ignored most of the limitations presented by scientists when referring to the expert evidence. Among the legal system trial participants, judges were the most accurate in summarizing the expert evidence. Approximately 80% of the expert scientific opinions on GSR transfer and persistence were presented in the form of the Probability of the Hypothesis (P(H|E)), rather than in the form of the Probability of the Evidence (P(E|H)), and without explicitly stating competing hypotheses. For DNA cases, where experts refrained from commenting due to a lack of empirical knowledge, lawyers seemed prompted to do so themselves. This resulted in instances of unsubstantiated claims put forward to the judge or jury. In terms of accuracy, both lawyers and judges showed strengths in discussing and arguing body fluid identification statements, but weaknesses in discussing and arguing negative results. These incorrect reflections seemed to stem from a lack of understanding of the meaning of the absence of DNA on evidence.

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### Transfer, Evidence, Expert

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## F8 An Evaluation of Expert Testimony on Secondary Transfer: Knowledge and Perceptions of Potential Jurors and Those Employed in the Criminal Justice System

*Rachel H. Oefelein, MSc\*, DNA Labs International, Deerfield Beach, FL 33441*

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**Learning Overview:** After attending this presentation, attendees will better understand how reporting statements and testimony are perceived by potential jurors as well as those working within the criminal justice system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees who want a better understanding on the perception, knowledge, and impact of expert testimony pertaining to secondary transfer and activity level propositions from the perspective of those involved in criminal justice as well as potential jurors.

Peruse any literature on secondary transfer or activity level propositions and nearly all authors will state there is a need for additional research, that in court the question is no longer whose Deoxyribonucleic Acid (DNA) is this, but rather how did it get here, and that those in the industry need to be able to say more. Additionally, it has been stated numerous times in scientific literature that if the forensic expert cannot speak to the significance of evidence, how is the court supposed to be able to speak to it? While conducting a literature review of existing literature on secondary transfer and activity level proposition, the common themes were noted throughout most current literature; however, there was no reference to data that quantified why these themes are important. An important question to answer when considering any research project has always been, is there a need for this research?

A 15-question survey comprised of demographic and DNA-based questions was created and published. Approval from Griffith University Research Ethics was obtained prior to initiating this project.<sup>1</sup> Demographic questions sought to obtain data on the gender, age, level of education, ethnicity, occupation, court system, and past jury duty service of respondents. The DNA questions explored the respondents' opinions on the importance of DNA, of knowing the source of DNA on evidence and the activity level propositions as well as their knowledge of secondary DNA transfer and examined thoughts on hypothetical reporting statements from four different mock case scenarios. The overall responses, as well as the responses plotted against the occupations of respondents, were evaluated.

It should be noted, this respondent group was recruited via LinkedIn® and email and as such, the population set was skewed toward those involved in the criminal justice system but also those that could serve as potential jurors. The respondents were primarily White American females that had completed at least some degree in higher education. Overall, the survey confirmed what much of the peer-reviewed literature states: there is a need for additional research on secondary transfer and activity level propositions, and that expert testimony is critical in ensuring that jurors are educated during testimony and understand reporting statements. Furthermore, it is critical that the scientists explain the findings to the court so they may more accurately understand the significance of evidence without overestimating or underestimating the results. What was surprising was the quantity of forensic scientists that also demonstrated a need for greater understanding on the topics of secondary transfer, activity level propositions, and the evidentiary value of different statistics. This presentation will explore the survey results in more detail and the implications of these results.

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<sup>1</sup>. Griffith University Research Ethics. Ethics reference number 2020/339.

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### Secondary Transfer, Activity Level Proposition, Expert Witness Testimony

## F9 Expert Witness Testimony and Adaptive Speech Devices: Feasible Reality or Impractical Liability?

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of bias and credibility challenges impacting expert witnesses who require Speech Generating Devices (SGDs) to communicate. This study examines the relationship between SGDs, juror perceptions of credibility, and attitudes toward experts requiring SGDs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining juror bias, promoting awareness of SGD technology available to current or future analysts and experts, fostering change within the justice system, and encouraging further research into this relatively uncharted topic.

Research in this area may prove relevant, not just to expert witnesses, but also to forensic analysts with disabilities given the implications of the Sixth Amendment's Confrontation Clause, *Melendez-Diaz v. Massachusetts*, and subsequent cases in which a forensic analyst is the expert witness at trial.<sup>1</sup>

Currently, courtrooms turn to expert witnesses with increasing frequency. Credibility is crucial for expert witness testimony and can significantly influence case outcomes. Technological advances have improved quality of life for many persons with complex communication needs. Persons with disabilities such as autism and Amyotrophic Lateral Sclerosis (ALS) can participate, with the help of SGDs in multiple facets of society that were previously inaccessible. Most research into SGDs has focused on persons with moderate to profound disabilities. However, advanced devices allow persons with speech-related disorders, disabilities, or injuries without intellectual impairment a path to communication in the community and the workplace. Unfortunately, SGDs have many limitations and use may not translate to the highly scrutinized and technical areas of forensic analysis and expert witness testimony, where life and death decisions are dependent, in part, upon credibility perceptions.

A survey was designed and participants were asked to watch two video clips of a natural speaker and an expert testifying with the use of an SGD. Participants continued by rating each expert's performance on a 20-item witness credibility scale.<sup>2</sup> The survey also included multiple follow-up questions in a yes/no/neutral response format involving attorney attitudes toward SGD use.

When comparing aggregate mean responses between both experts, Student *t*-test results provide compelling evidence to suggest overall credibility and two subscale measures of credibility, trustworthiness and knowledge, are compromised by the use of an SGD (*p*-value < .05). For attorney attitudes, three chi squared goodness of fit tests indicated significant differences between expected and observed response frequencies (*p*-value < .05). Further analyses of the "yes/no" questions with the neutral responses eliminated showed a significant preference for the verbal expert over the non-verbal expert using an SGD.

The outcome of the study indicated that expert witnesses testifying with an SGD will experience significant stumbling blocks in demonstrating credibility. However, as with any breakthrough in commonly held negative societal views, more research and greater awareness can reshape the future for persons with complex communication needs and those surrounding them.

### Reference(s):

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2. Brodsky, S.L., Griffin, M.P., and Cramer, R.J. (2010). The Witness Credibility Scale: An outcome measure for expert witness research. *Behavioral Sciences & the Law*, 28(6), 892–907. <https://doi.org/10.1002/bsl.917>.

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### Expert Witness Credibility, Speech Generating Devices, Disability Bias

## F10 A Double Truth: When Scientific and Judicial Conclusions Are Divergent

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**Learning Overview:** The goal of this presentation is to evaluate the correspondence between scientific evidence and the final judgment of the court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that scientific truth does not always correspond to procedural truth.

The present study compares three similar cases of suspected homicidal drowning in which the same complete methodological approach led to different court decisions.

The first case concerns the death of a 29-year-old girl found dead in the bathtub of her apartment. External examination of the body revealed bruising on the neck and forearms, conjunctival petechiae, and froth exuding from the mouth. The autopsy showed petechiae on temporalis muscles, epicardium, visceral pleura, hemorrhages of the neck muscles, and reddish froth in the trachea. Histological analyses showed zones of contraction-band necrosis in the myocardium, pulmonary edema, and emphysema. The immunohistochemical analysis showed positivity for anti-CD15, anti-tryptase, and anti-IL15 antibodies on neck muscles, confirming the vitality of neck lesions. The analysis of a sample of femoral bone marrow, using a phase-contrast microscope, showed the presence of diatoms. According to the evidence deriving from a complete methodological approach, death was attributed to drowning in a subject with vital neck compression signs. The court sentenced the boyfriend of the girl who confessed to having stunned the victim by strangling her in a fit of rage; he then placed the body in the bathtub to revive her. After realizing that the girl was dead, he left the apartment.

The second case refers to the death of a 23-year-old man found as a corpse in a river. The external examination showed the presence of abrasions and bruises of the face, lacerations of the labial mucosa, and fracture of the left upper incisor tooth. The autopsy showed the presence of subarachnoid hemorrhage, multiple rib fractures, laceration of an ileal loop with leakage of fecal material, multiple lacerations of the liver parenchyma, and hemorrhagic infiltration of the adipose capsule of the right kidney. Histological and immunohistochemical exams confirmed the vitality of the lesions. The diatom test was positive. Scientific evidence suggested that the victim had been beaten, then died by drowning. During the interrogation, the compatriots of the victim confirmed they had beaten the boy and then threw him into the river while he was still alive. In light of these declarations, the court sentenced them.

The third case is related to a 16-year-old girl found dead on the shores of a lake. The external examination of the corpse revealed froth exuding from the mouth and a linear excoriation on the left side of the neck. No other signs of external traumatic injury were found. The autopsy revealed sand in the pharynx and larynx and frothy fluid in the trachea and the main bronchi. Histological study of the heart showed diffuse foci of lymphomononuclear inflammatory infiltrates in the interstitial space with cytotoxic necrosis of myocells. Histological analysis of the skin sample of the neck lesion showed the absence of the epidermis and of hemorrhagic extravasations in the underlying layers; immunohistochemical examination revealed the absence of inflammatory cell infiltrates. This evidence suggested a postmortem production of the lesion. No diatoms were found in the bone marrow. Toxicological tests were negative for alcohol and drugs. The death was attributed to heart failure in a subject with active lymphocytic myocarditis. Despite the scientific evidence provided by the forensic pathologist, the judge requested the expertise of a pulmonologist. He claimed the death was due to drowning because of the presence of foam in the airways, even if it is a non-pathognomonic sign of drowning. The court sentenced the boyfriend of the victim, who was the last to see her alive. The final verdict of the judge ruled that the boy had killed the girl by holding her head underwater.

Forensic evidence is crucial in criminal investigations to reconstruct critical elements of a crime. The presented study shows that scientific evidence and judgments of the courts does not always coincide.

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### Scientific Evidence, Methodological Approach, Drowning



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## F11 Post-Conviction DNA Testing: A Two-Year Summary of a Law School and University Collaboration to Identify and Evaluate Post-Conviction Cases

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**Learning Overview:** After attending this presentation, attendees will understand the California Forensic Science Institute-Loyola Law School's 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 CFSI within the School of Criminal Justice and Criminalistics at California State University, Los Angeles, established a partnership with Loyola Law School's Loyola Project for the Innocent (LPI) 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 funding under the 2019 Postconviction Testing of DNA Evidence solicitation to provide critical assistance to individuals convicted of a serious felony that meets 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.

Since 1989, DNA testing has facilitated the exoneration of 511 individuals nationwide. Only 26 of 511 DNA-related exonerations have occurred in California—a state that incarcerates more inmates than any state other than Texas. Of the 26 cases, only six were in Los Angeles County, a county that produces nearly half of all serious felony convictions in the state. Under California's DNA testing statute (Penal Code §1405), a request for DNA testing must satisfy several criteria. These include a claim of innocence and 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 of 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.

This presentation provides a two-year overview of the CFSI-LPI case review model, emphasizing the identification and evaluation of cases that meet the criteria under CA Penal Code §1405 for post-conviction DNA testing. This summary will include the number of DNA cases reviewed and investigated; the crimes for which clients were convicted, including those that have a sexual assault component and/or resulted in the death of the victim(s), and the number of evidence searches conducted, specifying the cases where evidence was located versus destroyed or missing. Further, this summary will disclose the number of items submitted for Short Tandem Repeat (STR), Y-chromosomal STR (Y-STR), mitochondrial DNA (mtDNA) analysis, and whether a viable profile was obtained in addition to the probative value of the DNA profile (supporting versus refuting the post-conviction claim or inconclusive). This presentation will also specify the number of DNA profiles uploaded to the Combined DNA Index System (CODIS) versus the number of CODIS hits. It will conclude by describing obstacles to the project objectives throughout the grant award period 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.

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### Post-Conviction, DNA Testing, Law School-University Collaboration

## F12 The Application of Virtual Reality in Forensic Science

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**Learning Overview:** After attending this presentation, attendees will know how to apply the application of Virtual Reality (VR) to the crime scene investigation, reconstruction, and representation in the future court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the new technology, VR, applied in crime scene reconstruction. The resulting video and model could be a useful example for judicial education and could be presented at international conferences, showing how forensic science is being used in Taiwan. In the future, more technologies applied in the judiciary may develop a better environment for justice.

Due to the limitations of traditional evidence search tools, crime scene investigations are still using 2D evidence for documenting crime scenes. Compared with real or virtual 3D evidence for crime scenes, critical 2D evidence information may be lost. Solving this problem is an urgent need in forensics. Scanning 3D crime scenes, constructing 3D scenes of VR, and solving the difficulty of traditional 2D images to present the real situation of complex crime spaces with different perspectives allows observers to experience the realism of 3D spaces.

Using science and technology in investigations and trials is a trend in forensic science, leading to judicial reforms that safeguard justice. Advanced inspection technologies could significantly promote the effectiveness of decisions in forensic investigations. However, in the National Conference on Judicial Reform, a frequently mentioned issue is the lack of the education in forensic science in Taiwan. Developing a national committee on forensic science has thus been suggested to lead the promotion of the judicial experts' abilities in criminal investigation, to discover the truth, and to reduce miscarriages of justice. Judicial personnel are also actively cultivating science and technology in the courts, raising the public's trust in justice.

This study presents the application of VR on crime scene investigations, reconstructions, and future presentations in court. This presentation will focus on studying the case of Su Chien-Ho, one of the most famous cases in Taiwan. Three death row sentences were suggested as a miscarriage of justice and redressed because of the key issue: the space of the crime scene was too narrow to bear four criminal suspects' fierce attacks at the same time. This study intends to re-establish the basis of the 3D model crime scene through mapping software and on-site photos at the Taiwan Police College. This study attempts to apply VR and animation to represent the conditions of the crime scene and the scientific evidence. The 3D model and the animation of the crime scene in the case of Su Chien-Ho could effectively demonstrate the space, scientific evidence, and meaning of criminal scene reconstruction.

This research also aims to introduce 3D scanning, modeling, and other technologies to integrate VR into the field of crime scene investigation. By explaining the problems of traditional 2D recording of crime scenes, and emphasizing the use of 3D scanning technology, the effect of 3D comparison of evidence can be enhanced compared with the traditional spatial presentation of 2D crime scenes. There is no need to rely on imagination to build a 3D model quickly. It can also be equipped with VR glasses; if it is not convenient to visit the crime scene in person, investigators can observe various evidence of the crime scene in different perspectives. In the future, investigators can refer to the use of VR technology to reconstruct the scene of a criminal case from a flat file or photos for a VR that can be zoomed in, zoomed out, or moved according to the user's needs. This is more in line with the actual situations for criminal investigations in order to further improve the efficiency of case solutions.

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### Virtual Reality, 3D Modeling, Crime Scene Reconstruction

## F13 Transparency to the Defense Is Essential to Truly Pursue “Justice Through Truth In Evidence”: A Defense Perspective on Discovery Reform in New York and a Possible National Standard

Allison Lewis, JD\*, *The Legal Aid Society, New York, NY 10013*

**Learning Overview:** The goal of this presentation is to offer a defense perspective on why robust discovery laws that mandate specific laboratory disclosures could benefit all parties and the criminal justice system as a whole. After attending this presentation, attendees will gain some insight into defense priorities and how laboratory transparency is essential to the pursuit of “justice through truth in evidence.” In addition, attendees will be educated on the history, challenges, and lessons learned from the extensive discovery reform that took effect in New York in 2020. Finally, an additional goal of this presentation is to offer proposals for how the system may increase efficiency by moving from resistance thinking to acceptance and transparency in discovery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing all attendees—whether attorney, laboratory, or law enforcement—a greater understanding of how cooperation and full transparency could benefit all relevant stakeholders. This presentation will attempt to close the awareness gap that often divides parties over who should get what, when, and why. Finally, this presentation will propose new ways to streamline discovery from a defense perspective to ensure robust disclosures, building toward a new understanding between players often adversarial to one another.

**Hypothesis:** There can be no question that in the pursuit of “justice through truth in evidence,” a full commitment to providing comprehensive discovery to the defense is essential.<sup>1-3</sup> For the defense, this includes what may be considered standard or traditional DNA discovery items, like lab reports and basic data. But it also includes other less traditional items necessary to understanding that discovery. Where resources are limited and time is always of the essence, it may be tempting for a laboratory to prioritize efficiency and economy. However, justice is only truly served when full transparency and disclosure are a top priority.

Discovery regulations and practice vary across the country.<sup>4</sup> From a defense perspective, without the full force of clear statutory laws to mandate specific discovery in an adversarial system, the defense will continue to confront systemic resistance to efforts to obtain all necessary discovery. Without clear statutory guidance, the defense will often be called upon to justify each individual item of discovery requested. These piecemeal battles can often hamstring counsel, court, and the lab in inefficient and counterproductive ways. As an alternative, this presentation proposes new ways of thinking about DNA discovery to ensure justice is achieved.

As an example and cautionary tale, this presentation will examine the history and recent discovery reform in New York state.<sup>5</sup> For many years, the defense bar was forced to practice largely in the dark, under narrow discovery rules that came to be known as the “blindfold laws.”<sup>6</sup> For DNA discovery in New York City, the defense fought repeatedly over the relevance and necessity of various material from the Office of the Chief Medical Examiner (OCME), creating lengthy and contentious delays in court proceedings. Prosecutors routinely opposed providing these materials, arguing that providing various materials were burdensome, excessively time consuming, even warning of possible defense manipulation of data.<sup>7</sup> Some progress was made, but it was unnecessarily slow and difficult, with many judges denying access for varying reasons.<sup>8</sup> Once the reform took effect, discovery obligations changed drastically overnight. This presentation will specifically address the myriad ways these statutory changes have affected discovery practice in New York City, examine the history of this change, and discuss the challenges and victories that have come along with it.

### Reference(s):

1. *Brady v. Maryland*, 373 U.S. 83 (1963).
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3. Janet Moore. *Democracy and Criminal Discovery Reform After Connick and Garcetti*. 77 Brook. L. Rev. 1329 (2012).
4. *Expanded Discovery in Criminal Cases: A Policy Review*. Published by The Justice Project, avail. at [https://www.pewtrusts.org/~/media/legacy/uploadedfiles/wwwpewtrustsorg/reports/death\\_penalty\\_reform/expanded20discovery20policy20brief.pdf](https://www.pewtrusts.org/~/media/legacy/uploadedfiles/wwwpewtrustsorg/reports/death_penalty_reform/expanded20discovery20policy20brief.pdf).
5. CPL §245.20.
6. Ashley Southall and Jan Ransom. Once as Pro-Prosecution as Any Red State, New York Makes a Big Shift on Trials. *New York Times*, May 2, 2019.
7. *People v. Stover*, Ind. No. 1512/2015 (filings and decision, March 16, 2016 (Stolz, J.)).
8. *People v. Mohammed*, 52 Misc.3d 242, May 6, 2016 (Sup. Ct., Bx Cty) (Barrett, J.).

### Transparency, Criminal Defense, DNA Discovery Standards



## F14 Protecting the Innocent: When and Under What Circumstances Should Source-Level DNA Evidence Be Admitted in Cases Where the Relevant Issue Is “Activity” Rather Than “Source”?

*John D. Schmid, JD\*, Sixth District Public Defender, Duluth, MN 55802*

**Learning Overview:** After attending this presentation, attendees will appreciate the significant need for further research into the presentation of “source-” and “activity-” level DNA evidence and whether additional safeguards are necessary to ensure that the information is not misconstrued by jurors, judges, or litigants. This presentation will also encourage researchers with technical expertise and resources to study whether existing procedural safeguards are sufficient to protect innocent defendants faced with incriminating DNA evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness to practitioners about the potential for testimony and statistical statements regarding source-level DNA evidence to be misconstrued. This presentation will also explore whether existing procedural safeguards, such as cross-examination and evidentiary rules, are sufficient to protect innocent defendants when the prosecution presents incriminating DNA evidence.

Current DNA testing kits are incredibly sensitive. There is now a substantial risk that an innocent person’s DNA will be detected in a crime-scene stain. As a result, the relevant question in many cases with DNA evidence is not whether the defendant is the source of DNA in a crime scene stain, but how did the defendant’s DNA come to be where it was found.

The risk of wrongful convictions in cases where the relevant question relates to “activity” rather than “source” is compounded by two additional factors. First, fact finders frequently misinterpret forensic statistical evidence. For example, mock juror studies have found that laypersons tend to “transpose the conditional” when the weight of an association is presented as a likelihood ratio. Second, presenting statistical evidence that strongly associates the defendant with DNA in a crime scene stain creates a real risk that fact finders will improperly carry over the source-level statistic to higher-level propositions, like activity or offense.

Preventing wrongful convictions when the relevant question surrounding DNA evidence is “activity” rather than “source” presents significant challenges to everyone in the criminal justice system. This presentation will discuss the Farah Jama case as one example of how such challenges arise. It will also explore how analyses of wrongful convictions and mock juror studies can help us rise to meet these challenges. A robust body of innocence research has identified misleading and/or overstated forensic testimony as a major contributor to wrongful convictions. The ability of existing procedural safeguards to protect innocent defendants faced with incriminating forensic evidence is less understood.

Mock juror studies provide a useful method to assess the impact of procedural safeguards in a criminal trial. Future research should include mock juror studies with fact scenarios involving the following three constants. First, ground truth in each scenario must be actual innocence. Second, the evidence presented to mock jurors must include a strong statistical association between the defendant and DNA in a crime scene stain. Third, the relevant issue must involve activity rather than source. Such scenarios can be derived from real-world cases or from laboratory studies involving secondary transfer and/or contamination.

Judges are frequently called upon to assess the potential for prejudice created by the prosecution’s introduction of forensic testimony, including DNA evidence. Such decisions are far too often based on anecdote and intuition rather than data. The materials discussed in this presentation will provide empirical guidance to practitioners and decision makers in cases where the relevant issue is activity rather than source. It will also provide direction for future research that can be readily applied to cases where the prosecution offers source-level DNA evidence against the accused.

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### DNA, Activity, Source





## **F15 Ineffective Assistance of Counsel in DNA Cases: Lawyers and Judges Don't Get It**

*Albert (Buzz) E. Scherr, JD\*, University of New Hampshire School of Law, Concord, NH 03301*

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**WITHDRAWN**

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## F16 Partnering With Law Enforcement to Implement Novel DNA Technologies

*Andrew Singer\*, Bode Cellmark Forensics, Lorton, VA 22079*

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**Learning Overview:** After attending this presentation, attendees will understand how partnering with law enforcement to evaluate and adopt technologies can lead to impactful implementation.

**Impact on the Forensic Science Community:** This presentation will benefit the forensic science community by showing how approaching new technologies collaboratively can create a model for evaluating and implementing new technologies in the future.

Every year, new technologies are introduced that have the potential to change the way we do things. Some are new applications of technologies from other spaces, some are novel technologies that can significantly change or disrupt the way we operate. Not every individual, company, or agency is going to be an early adopter. Many do not have the funding, the bandwidth, or the desire to implement these technologies. However, it is important to support early adopters or we, as a community, risk the technology becoming obsolete or discarded before it has the opportunity to be impactful and make a difference to our communities.

Bode began working with law enforcement agencies to develop local DNA databases in 2013 to address a specific need. Small and midsize departments often do not have the same access to technologies that larger agencies have. Through the local DNA database program, DNA results are delivered from both reference samples and evidence in 30 days or less. Additionally, with the wide variety of DNA legislation, many state DNA databases do not contain DNA profiles from arrestees. Approximately 40% of states do not take DNA upon arrest, meaning the databases are only populated with those that are convicted of crimes, and most often that is further limited to felony convictions. As a result, the impact of DNA technology to drive or support investigations is limited in higher volume property or gun crimes, which are often the most common in the department's community. Local databases enable law enforcement to use DNA on the cases impacting their community the most.

Throughout the development of DNA programs for law enforcement, Bode has regularly engaged with clients and stakeholders, both individually and as a group. There are no universal standards specific to local databases, but most of the stakeholders have an interest in ensuring the technology is properly adopted so that it protects the program and allows it to develop.

Bode took that approach with its clients, including creating regular working groups, sharing protocols, and developing guidelines to manage local DNA database programs. As an organization of forensic experts, it was important to share knowledge with those using it so that they understood both the power and limitations of how it could help. By working and engaging with clients, they also took ownership of the technology and wanted to protect the work.

As new technologies such as rapid DNA are introduced, ongoing and regular engagement with these stakeholders is essential. Not every technology benefits the organization, but it is also understood that helping to implement new solutions rather than ignoring them would ultimately benefit the client, their communities, and the technology itself. Specifically, for rapid DNA, this meant offering a turnkey solution—validating the technology, creating guidelines for use, and designing training programs.

We all share a mission of improving public safety. All of us can continue to embrace that mission through using the tools that are available. Through this collaboration, not only have we enabled new technology to help get answers sooner, but we have also created a template for introducing new technologies in the future.

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### DNA, Rapid DNA, Database



## F17 The Implementation of Rapid DNA: Prevent Tomorrow's Victim

*Frederick Harran, MS\*, Bensalem Township Police Department, Bensalem, PA 19020*

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**Learning Overview:** After attending this presentation, attendees will understand how a law enforcement agency can utilize DNA technology locally to develop investigative leads while maintaining a forensic standard for testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing valuable data information on how a law enforcement agency can adopt new technologies and apply them to their operations properly.

The Bensalem Township Police Department (Bensalem, PA) will introduce their program that created a local DNA database through partnership with an accredited laboratory and the use of Rapid DNA technology to develop real-time investigative information. Bensalem was the first police department in the country to utilize a Rapid DNA instrument in conjunction with its well-established local DNA database.

Through an introduction that includes a background of the community, this presentation will outline Bensalem's approach to solving cases, and more importantly preventing crime. This presentation will review actual case examples to demonstrate the impact of the program on multiple crime types. Additionally, goals in the development of the program itself and how that has evolved over time will be discussed.

Best practices for implementing and using the technology to maximize the impact on the judicial system will be discussed. Over the course of almost ten years of the program, Bensalem has regularly introduced new, cutting edge technology to help support their mission of improving public safety. As a result, Bensalem has had to regularly introduce and change operational procedures and training programs to ensure their program meets standards expected by the courts. Their program has also grown regionally, and, as a result, Bensalem has regularly engaged with neighboring departments to support their implementation and ensure the integrity of the program is maintained. Both the quality and the impact of the program are regularly monitored.

The development of the local DNA database has included various stakeholders, all with various levels of buy-in and expectations. This included internal buy-in through multiple divisions within the police department, such as the criminal investigations division, the narcotics division, and patrol officers. Outside of the organization, it was important to involve the prosecutors and local government as well as the community. Without complete buy-in, risk for misuse or lack of quality could occur.

The results of the program have generated a significant reduction in local crime, including reducing burglary crime by more than 40% in the first year of the program alone. The public-private partnership has enabled this technology to be quickly adopted while having a significant, positive impact on the community. The program has also had a positive return-on-investment as well when compared to other solutions to reduce and prevent crime, with the annual investment in the technology being far less than the cost to put a senior patrol officer on the street.

Overall, attendees will understand how advanced DNA technology can be used successfully while meeting quality standards to maintain DNA as the gold standard for forensic investigations.

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### DNA, Rapid DNA, Database

## F18 When Bad Science Leads to Incorrect Guilty Sentences: Reconsidering the Lorandi-Bugna Case

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**Learning Overview:** After attending this presentation, attendees will have learned how a cold case, in which scientific evidence played a key role in arriving at a life sentence, can be reconsidered under the lens of more recent developments in science that prove how the original scientific evidence was destroyed by an improper manipulation and bad science tried to reconstruct it, without detecting important contradictions that may have exonerated the defendant.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how a thorough critical analysis of scientific evidence can detect contradictions that undermine that very evidence. This presentation will build competence on how to understand whether the technical witnesses have correctly analyzed the objects on which scientific evidence had focused or whether the pressure put on them by the prosecutor had biased them to the extent that they could make macroscopic, undetected errors. This presentation will also impact attendees by showing how advancements in science may bring out new and different evidence.

The Lorandi-Bugna case is an Italian cold case that goes back to February 2007, when Clara Bugna, a 54-year-old woman was murdered in her home in a small village near Brescia in northern Italy. She was found strangled in her apartment, with the door locked (Clara's keys were never found). An iron was found turned on, a couple of freshly ironed shirts were discovered, and a half-ironed short was on the ironing board.

The murder was discovered because Clara's employer (the owner of a restaurant where Clara worked as a cook), alarmed since she did not show up, called her husband, Bruno Lorandi, who was at work, and they discovered the corpse.

Bruno Lorandi was suspected of having murdered his wife, despite claiming his innocence. He was accused of having staged the crime scene after having killed Clara. Therefore, the investigation focused on assessing whether the iron was switched on before or after he left the apartment to go to work.

To this purpose, the electric power company was asked to provide the energy consumption measured by the electric meter, and the iron was tested to compare its electrical consumption with the energy measured by the electric meter. The electric meter was a new-generation (at that time) electronic smart meter that could be read remotely, though the remote access routing had not yet been thoroughly tested. An attempt at remotely reading the counter was made, then aborted due to a problem on the communication line. It was discovered that, due to an undetected bug in the meter software, an aborted reading operation caused the internal clock to lose its alignment, so the stored energy values could not be attributed to the corresponding time. A second attempt was made and was also aborted, which worsened the situation.

Despite, due to this bug, the internal data being fatally corrupted, the expert witnesses charged with the electric energy consumption analysis by the prosecutor claimed to have succeeded in reassigning the energy consumption to the correct time and, without considering the high measurement uncertainty in their tests, concluded that the iron was switched on a few minutes before Bruno left the apartment. Bruno was sentenced to life imprisonment and the sentence was confirmed by the appeal court and the Italian Supreme Court.

Recently, a new technical analysis of the energy consumption, based on more recent analysis techniques, discovered a blatant incongruity in the original energy reconstruction: a consumption of several kWh for about four hours was assigned to a day, after the murder was discovered, when the apartment was definitely empty and all loads were switched off as the apartment itself was sealed off by the police. This casts a more-than-reasonable doubt on the validity of the reconstruction.

Based on this, Lorandi requested his case to be reconsidered and a decision is pending at the time this abstract was submitted. Regardless of the conclusion, this case appears to be very instructive because it shows how a superficial analysis of strongly corrupted evidence may lead to wrong conclusions.

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### Bad Science, Corrupted Evidence, Innocence



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## F19 The Need for Ethical, Legal, and Social Implications (ELSI) Evaluations in Forensic Science Methods and Police Investigative Technologies

*Sarah Chu, MS\*, Innocence Project, New York, NY 10013*

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**Learning Overview:** After attending this session, attendees will better understand the essential need to assess the ethical, legal, and social impacts of forensic methods and investigative technologies prior to their implementation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by recommending policies that can help improve the ethical implementation of forensic science methods and police investigative technologies.

When the National Human Genome Research Institute (NHGRI) was established in the National Institutes of Health, Congress also established the Ethical, Legal, and Social Implications (ELSI) Program as a companion program. The National Institutes of Health Revitalization Act of 1993 mandated that not less than five percent of the NHGRI budget be focused on ELSI research to ensure that as DNA technology advanced, cohorts of bioethicists would be cultivated to be able to answer the pressing problems that DNA technology would present. However, ELSI provisions were never attached to the development of other technologies serving society.

Today, we are in the midst of a technological revolution. DNA profiles can be developed in 90 minutes, police departments can pay private companies to store DNA profiles taken “voluntarily” from arrestees or by DNA dragnets, and sanctioned state DNA databases are being leveraged to non-consensually expand their reach to sift through innocent people in search of an unknown person responsible for committing a crime. Much of the ELSI research programs have focused on biomedical issues and, while experts have raised privacy and racial justice issues with the new uses of state DNA databases, the criminal legal system has pressed forward in supplying law enforcement with investigative technologies without due regard to their validity, reliability, efficiency, and just application. Notably, we have witnessed the national uproar to the use of facial recognition technology and witnessed the first known wrongful arrests attributed to its use. Communities across the country have expressed their concerns about the application of an unreliable technology, its differential impact in both use and application on communities of color, as well as its impact on their rights to a private life.

It has become clear that simply evaluating the scientific validity and reliability of forensic science methods and police investigative technologies is insufficient for the real-life implementation of these tools in a flawed and racially biased criminal legal system. This presentation will identify a few examples of forensic science and police investigative technologies, discuss their ELSI implications, and present policy recommendations to evaluate their valid, reliable, and just application. These policy recommendations will include a special focus on how structural inequalities can contribute to racial disparities in how the benefits and consequences of technologies are distributed. As a community, we must contemplate and address the meaning of our use of forensic science methods and police investigative technologies as applied in the context of our existing criminal justice system. It is only then that science can truly advance justice for all.

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**Ethics, Social Implications, Racial Disparities**



## F20 Digital Evidence in Criminal Cases Before the United States Courts of Appeal: A Follow-Up Study on Trends and Issues for Consideration

*Martin Novak, MPA\*, National Institute of Justice, Washington, DC 20531*

**Learning Overview:** After attending this presentation, attendees will: (1) understand the most relevant legal issues related to digital evidence, (2) understand precedent cases that impact digital evidence before the courts, (3) understand the most prevalent challenges to digital forensics, and (4) will discuss challenges on the horizon for digital evidence in criminal cases. Overall, attendees of this session will be informed as to how digital evidence has withstood challenges in appeals of criminal cases before the United States Courts of Appeal.

**Impact on the Forensic Science Community:** The current study is a follow-up to an American Academy of Forensic Sciences 2020 presentation.<sup>1</sup> The current study examines appeals of criminal cases before the United States Courts of Appeal from January 2016 through June 2020, where one or more appeal claims were related to digital evidence. The purpose of this research was to determine if the legal landscape has changed since 2015; examine the most relevant legal issues related to digital evidence; and analyze how precedent cases may have affected digital forensics as evidence.<sup>2-5</sup>

We live in a more connected world today than we did five years ago. Wearable devices have made their way into court as evidence recently, though their ultimate disposition as evidence is yet to be determined. Other important issues before the courts since 2015 include whether reasonable suspicion is necessary for an intrusive digital search at our nation's borders; whether compelling a suspect to provide their unlock code is "testimonial" for purposes of Fifth Amendment protection; the scope and particularity of digital search warrants when the government uses Network Investigative Techniques in online investigations, and finally whether the use of Cell Site Location Information (CSLI) for geo-location constitutes a search.

This analysis was based on a review of cases in the United States Circuit Courts of Appeals from 2016 through June 2020. Cases were identified via LexisNexis, using the following search terms: Probable Cause, Encryption, GPS, Geolocation, Geo-Fence, Onion Router, Wearables, Internet of Things, Text Message, Cryptocurrency, Network Investigative Tool (NIT), Particularity, Cell Phone, Metadata, Digital Evidence, Dark Web, ECPA, Social Media, and Child Pornography. Results include 80 criminal appeals before the United States Courts of Appeal. Of those cases, 88.75% were affirmed for the government. Offenses included possession and/or distribution of child pornography; narcotics possession or distribution; illegal weapons possession; armed robbery; sex crimes; violent offenses; and white collar crimes. The most frequently occurring basis for appeal was probable cause, followed by sufficiency of evidence, scientific merit, authenticity, and relevancy. In addition to the results, this presentation will discuss what has changed since 2015.

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5. *United States v. Kolsuz*, 890 F.3d 133 (4th Cir. May 9, 2018).

### Digital Evidence, Compelled Decryption, Geo-Location

## F21 Terrestrial Light Detection And Ranging (LiDAR) Scanning (TLS), Admissibility of 3D “Point Cloud” Evidence and Testimony

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**Learning Overview:** The goal of this presentation is to provoke thinking about the admissibility requirements for TLS both foundationally and as applied. There have been reported decisions regarding 2D LiDAR (for speed and distance) but almost none regarding TLS 3D “point cloud,” either as demonstrative evidence or a basis for expert testimony.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping lawyers, judges, and experts (e.g., engineers, accident deconstructionists, crime scene evaluators) prepare to proffer TLS “point cloud” evidence or to critique or oppose such evidence if it is not established in the particular case to have foundational validity and validity as applied.

The purpose of this presentation is to stimulate thinking about the validity and limitations of Terrestrial LiDAR Scanning (TLS) for forensic purposes and to address the admissibility of TLS as a basis for demonstrative evidence or expert testimony in the trial court. It is the hypothesis of this presentation that the validity of TLS technology has been established in the scientific community and foundational validity can be established as to an individual vendor’s products. The applications of TLS used to create demonstrative evidence or used as a basis for an expert opinion will require an individualized determination of admissibility and scope.

TLS is based on light detection and ranging technology, hence the name, LiDAR. It is a form of laser technology in which a target is illuminated with laser light. The target reflects the light, and it is captured by a laser scanning device. The scanning device derives complex geometric data from the reflected light, which is interpreted as a 3D representation of the target. When deployed at a scene, it can be used to create a “point cloud” which, in turn, can be interpreted as a 3D representation of an expansive area.

LiDAR itself is not a new technology as it relates to either speed computations or 2D computations relating to position or distance. For instance, LiDAR devices are used in lieu of the hand-held Radio Detection And Ranging (RADAR) devices for traffic police to “clock” the speed of motorists. LiDAR has also been used to determine the distance from one point to another where a roll-a-tape may be inconvenient, for instance, in determining whether a sex offender’s residence is within the prohibited distance from a school.

TLS, used to create 3-D reconstructions, has been used for scientific research, primarily in archeological, architectural, topographical, or geographical studies. TLS is newer in its forensic applications but has been used to create 3D models or computer recreations for demonstrative evidence or as a basis for an expert opinion at trial. Oddly, TLS, unlike 2D LiDAR, has seldom been reviewed for its foundational validity or validity as applied in significant reported decisions.

This presentation will review the cases in which TLS has been discussed as well as some of the issues that should be addressed in motions *in limine* to determine admissibility and the scope of demonstrative evidence or testimony based on TLS. Work is being done on both the foundational validity and validity as applied of TLS. For instance, the National Institute of Justice (NIJ) Working Group for Criminal Justice Applications issued its first report on TLS in July 2020 that recognized that there are “no standardized, vendor agnostic guidelines” for criminal justice applications of TLS.

Meanwhile, TLS continues to be used in criminal and civil litigation at the trial court level. Even after the NIJ or other “best practices” are developed, trial judges—with the guidance of lawyers proffering the evidence and lawyers opposing it—will continue to have to make determinations as the “gatekeeper,” case by case. The questions of validity will have to be litigated and determined as to whether TLS foundationally or as applied meets the requirements of *Daubert* and *Kumho Tire*, as well as Federal Rules of Evidence 702, 703 and 403 (and their state analogs).<sup>1,2</sup>

It will be the conclusion of this presentation that TLS is fundamentally reliable as a scientific technology, although proprietary algorithms may have to be disclosed in order to evaluate the validity of a particular vendor’s product. There is peer-reviewed literature on TLS in general, and there is the ability to do “black box” proficiency studies of a particular vendor’s instrument. The bigger issue in actual litigation will be the validity of TLS as applied. Before TLS is allowed, either in the creation of demonstrative evidence or as a basis for an expert’s opinion, the trial court will have to make an individualized determination that the application meets the proper scientific standards and that the final evidence is of assistance to and not unduly prejudicial to the jury.

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### LiDAR, Admissibility, Point Cloud

## F22 Cell Phones Are the New DNA: The Emerging Role of Mobile Device Forensics in Wrongful Conviction Exonerations

*John J. Carney, Esq., JD\*, Carney Forensics, Scandia, MN 55073*

**Learning Overview:** New evidence produced from testing using new, innovative technology can open the door to judicial action for wrongful conviction review and exonerations. For decades, DNA testing was the most popular and effective example of new technology. Now mobile device forensics is emerging as an effective alternative to watch, or even the most promising form of new evidence, because of its speed of technology innovation and adoption and its support of a diversified number of proof vectors as compared to DNA. This presentation will compare and contrast mobile with DNA evidence. The goal of this presentation is to focus on effectively using mobile device forensics to recover new evidence in wrongful conviction challenges.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of competence (ability) and performance (action) by showing the practical value of technology innovation, whether biological or digital, in modern-day criminal justice in the United States and how to recognize opportunities to exploit it. This presentation will educate attendees on how to obtain new trials and exonerations based on effectively using sound digital forensic methods, best practices, and mobile device forensic tools designed to seek truth and defend the innocent.

The hypothesis or proposition of this presentation centers on how new technology and rapidly changing methods and tools provide a steady stream of opportunities for the Innocence Project, appellate public defenders, and private criminal defense attorneys to recover exonerating evidence in wrongful conviction challenges using advanced mobile device forensic science.

This presentation will compare and contrast mobile with DNA evidence. Like DNA, mobile may play a key role in wrongful conviction cases in the United States. (1) Technology-based innovation may provide new evidence required to open the door to wrongful conviction review. (2) Both methods have wide applicability for all persons in the population. (3) Everyone has DNA and most people have a cell phone. (4) The frequency of relevant and probative evidence recovery upon which cases turn compared to other forms. (5) Both have destructive use cases: DNA sample consumption; device chip-off; iPhone® jailbreaks; and Android® rooting.

Differences between mobile and DNA evidence may support mobile as the most promising form of new evidence. (1) DNA evidence is now regularly tested before trial. Many old cases have been reviewed using DNA. (2) Greater pace and rapid adoption for mobile technology innovation. (3) Diversified proof vectors for mobile as opposed to binary outcomes for DNA. (4) Material advantages of wrongfully convicted defendant's cooperation (personal knowledge) for mobile credentials. (5) Range of training and proficiency of mobile device forensic practitioners compared to DNA labs. (6) Limited time budgets and quotas for mobile device forensic practitioners in law enforcement. (7) Emerging awareness of mobile device evidence within the criminal justice community.

This presentation will focus on effectively using mobile device forensics to recover new evidence in wrongful conviction challenges. (1) Mobile device artifacts like device locations, contacts, text messages, chat, voice calls and messages, photos, videos, health and activity data, internet browsing, Google® searches, and mobile app evidence. (2) Pattern-of-life evidence showing insights into digital behaviors and frequency and location of routines.

This presentation will highlight opportunities to leverage mobile device forensics during the post-appeal time window to recover new evidence to qualify for reduced standards of proof. (1) Older feature phones with impaired device connections, or examined with primitive screen photographs instead of probative mobile device forensic tools, and advanced, deeply probative hardware extraction techniques like JTAG, chip-off, and In-System Programming (ISP). (2) iPhones® now support forensically sound, temporary jailbreaks which yield full file system extractions and deeply probative, newly discovered forms of mobile evidence and abundant deleted evidence. (3) Android® smart phones support forensically sound bootloaders and decrypting imaging methods that yield physical and full file system extractions and deeply probative, newly discovered forms of mobile evidence and abundant deleted evidence.

This presentation will conclude with a brief review of a successful wrongful conviction case study of a first-degree murder with a Life Without Parole (LWOP) sentence in Minnesota where an advanced mobile device forensic examination of the victim's feature phone from March 2008 recovered new, material evidence that contradicted eyewitness testimony and provided the wrongfully convicted defendant a new trial.

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### Wrongful Conviction, Mobile Device Forensics, DNA





**F23 #DataStories**

*Paul Reedy\*, 4th Street Global, Washington, DC 20003*

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**WITHDRAWN**

## F24 Is Epigenetics Ready for Prime Time? The Potential of Using DNA Methylation Patterns to Differentiate Monozygotic Twins and to Estimate DNA Donor Age

Robert F. Hedges, JD\*, Special Assistant Attorney General, Ann Arbor, MI 48104

**Learning Overview:** After attending this presentation, attendees will understand the potential for using epigenetic science in the form of DNA methylation patterns as forensic evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by summarizing the scientific literature on DNA methylation studies as it relates to distinguishing monozygotic twins and determining DNA donor age and addressing whether the present state of the research is sufficient to meet *Daubert* or *Frye* standards for courtroom admissibility.

Forensic DNA testing was first used in the 1980s and has since revolutionized forensic science. There have been tremendous advances in DNA technology through the decades, including Polymerase Chain Reaction (PCR) amplification and probabilistic genotyping, yet there remains limits to what traditional DNA testing can do. Among those are: (1) differentiating between monozygotic twins, and (2) estimating the age of a DNA donor.

The discipline of epigenetics offers a potential answer to these questions that may be on the cusp of qualifying for courtroom admissibility. The *Oxford Dictionary* defines epigenetics as “[t]he study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.” “Epi-” is a Greek prefix meaning “above,” “upon,” or “in addition to.” Here, this presentation will be looking at some of the potential information that can be found in a DNA sample “in addition to” the autosomal DNA profile.

The focus of this presentation will be on one area of epigenetics—DNA methylation. DNA methylation means the addition of methyl groups (CH<sub>3</sub>) to the DNA molecule. The methyl groups do not change the DNA code or sequence, but they change the way the DNA is expressed. Stated another way, DNA methylation results in gene silencing. This is necessary for normal growth and development, but can also affect disease progression.<sup>1,2</sup>

Of the four DNA bases, traditional dogma holds that only Cytosine (C) can be methylated in mammals—recent research suggests that Adenine (A) can also be methylated; thus nearly all of the scientific research has involved cytosine.<sup>3,4</sup> When cytosine is methylated it becomes 5-methylcytosine (5-mC). Most 5-mCs are found adjacent to Guanine (G) bases, which are called “CpG sites.” Clusters of CpG sites are called “CpG islands.” A test called “bisulfite sequencing” can be used to develop a DNA methylation profile. When DNA is processed using sodium bisulfite, methylated cytosine remains cytosine, while unmethylated cytosine changes to uracil, thus making it relatively easy to map DNA methylation patterns via conventional sequencing methods.<sup>1,2,5</sup>

The scientific literature on DNA methylation is extensive and goes back more than a half century. In 1945, scientist Rollin Hotchkiss described his findings on the methylation of cytosine into 5-methylcytosine. In the 1970s, Sir Adrian Bird described the role DNA methylation plays in gene transcription. The bisulfite sequencing test was invented in the 1990s by Marianne Frommer and Susan Clark and is considered the gold standard of DNA methylation analysis.<sup>1,5</sup> Over the decades, numerous studies, many of them involving twins, have reported findings based upon DNA methylation. Therefore, there exists a mature, extensive body of scientific research involving DNA methylation and, in recent years, some studies have specifically discussed the potential of DNA methylation science as forensic evidence.<sup>6,7</sup>

Several research papers reporting the ability to differentiate monozygotic twins using DNA methylation patterns will be discussed.<sup>8-17</sup> The extent and quality of the research will be examined with an eye toward whether they appear sufficient to meet *Dauber* or *Frye* standards.<sup>18,19</sup>

The case of *Commonwealth v Dwayne McNair* will also be reviewed.<sup>20</sup> In *McNair*, the prosecution attempted to use a different type of DNA test to distinguish monozygotic twins.<sup>20</sup> Specifically, the prosecution attempted to use ultra-deep next generation sequencing to search for somatic mutations.<sup>21</sup> The cost of the test was reportedly \$120,000. In April 2017, the trial judge ruled that such testing does not meet *Daubert* standards for admissibility. The decision was not appealed, but the prosecution prevailed at trial based on other evidence.

A significant number of recent research papers report the ability to estimate DNA donor age to a range of about 3.5 to 7 years based on DNA methylation patterns.<sup>22-33</sup> Such evidence could be used as investigative leads or settle questions of possible lab contamination in certain DNA-based cold cases.

Finally, this presentation will conclude by discussing a 2018 paper by Vidaki and Kayser that discusses in detail the state of DNA methylation research and the authors’ recommendations for further validation testing.<sup>34</sup> Further reference will be made to a similar Webinar by Vidaki and Lee recorded in 2017.<sup>35</sup>

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## Epigenetics, DNA Methylation, DNA Donor Age

## F25 Cheiloscropy: Lip Print Prevarications

Michelle L. Behan, JD\*, *The Behan Law Group, Tucson, AZ 85705-7746*; Gil Sapir, JD, *Chicago, IL 60680*

**Learning Overview:** After attending this presentation, attendees will understand that cheiloscropy lacks scientific evidential value.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of cheiloscropy's non-compliance with scientific and evidential standards.

Cheiloscropy in forensic science is the comparison use of lip prints and impressions to known exemplars for personal identification in the judicial system. Architectural pattern wrinkles and grooves on lips are used for determining individual characteristics with its appearance varying according to pressure, direction, anatomical surface, and the method of creation (similar to a fingerprint). Lip prints can be found on paper, clothes, glass, cigarette butts, and other surfaces resulting in variations of recovery and analysis.

Cheiloscropy initially began as a purported method of identification around 1970.<sup>1</sup> A survey inquiry of scientific literature from 1993, when *Daubert v. Merrill Dow Pharmaceuticals, Inc.* announced the judicial standards for scientific evidence, is presented for an overview assessment of cheiloscropy in human forensics.<sup>2,3</sup> Publications are often regionally from India.<sup>4</sup> The publications generally rely on erroneous statements and dated material and omit relevant judicial decisions, thereby presenting a veneer of credibility.<sup>2,5</sup>

Applicable standards and criteria for credible research are promulgated by the scientific community. Principles of metrology, including reproducibility and reliability, are necessary tenets of trustworthy science. In order to establish reliability, uncertainty of measurement and known error (random, systemic/procedural arithmetic, gross and confirmation bias) are required. Standardization of methodology is necessary for reproducibility.<sup>6</sup> Confirmatory DNA testing increases the test results reliability.<sup>7</sup> Unbiased peer-reviewed publication in respectable journals containing current research is necessary to establish reliability of methodology and results.<sup>8</sup>

Scientific evidence is derived from a process governed by the scientific method. Forensic science is the application of science to law. Forensic science is the most persuasive of all evidence. Reliable scientific evidence is essential for conviction and exoneration. Under *Daubert*, judges decide whether the discipline is reliable and admissible.<sup>3</sup> The 2009 National Academy of Sciences Report made numerous recommendations for objectivity and accountability, including standardization, rules governing courtroom scientific evidence, and pattern impressions.<sup>9</sup>

“ ‘Junk science’ is the mirror image of real science, with much of the same form but none of the same substance.”<sup>10</sup> Junk science or pseudo-science usually consists of flawed, unreliable, exaggerated, and sometimes fabricated testimony routinely founded on questionable methodology. Misrepresentation is a basis for publication retractions.

Proponents of cheiloscropy claim imprints are comparable to known exemplars for individual identification due their “unique” characteristics. However, the distinctiveness of an individual’s lip prints fails scientific and legal scrutiny.<sup>2,3</sup>

Current literature has adduced the following sample lip print prevarications: (1) focuses generally on anthropological topics; (2) reliance on outdated references from non-primary sources; (3) supposed uniqueness of labial wrinkles and grooves; (4) methodology lacks validity and reliability; (5) lacks current standards and techniques; (6) absence of method validation and quality assurance; (7) erroneously quotes reference sources; (8) lacks confirmatory salivary DNA analysis; (9) subjective pattern identification complications (guesswork, speculation, and bias); (10) non-existent forensic metrological analysis (uncertainty and potential error rate); (11) data integrity appears incompatible with modern tenets of “good science;” (12) paradigm conflicts exist (current scientific and legal standards); (13) ignores severe criticisms of the 2009 National Academy of Science Report on pattern evidence; and (14) disregards wrongful conviction where “phony forensic testimony” regarding cheiloscropy was a pivotal component.<sup>5</sup>

The American Bar Association has a post-conviction relief resolution to challenge convictions based on discredited forensic science.<sup>11</sup> California and Texas have post-conviction statutes for contesting convictions based upon false evidence, debunked science, or repudiated expert witness opinions.<sup>12-15</sup> The lack of judicial acumen and attorney competency regarding scientific evidential standards fosters unreliable (phony) scientific testimony and perpetuates wrongful convictions.

The scientific accuracy and legal reliability of cheiloscropy identification are not validated in current scientific literature. Accordingly, cheiloscropy should be considered pseudoscience or junk science.

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## **Cheiloscopy, Lip Prints, Junk Science**

## F26 Bayes' Theorem, Forensic Science, and the Law: Long-Lost Relatives or Feuding Family?

*Tim Kalafut, PhD\*, Sam Houston State University Department of Forensic Science, Huntsville, TX 77340*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of Bayes' theory and the presentation of DNA evidence at trial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting an awareness of how the findings and testimony of an expert can fit into the overall presentation of the evidence in the case.

The past several years have seen widespread adoption of the Likelihood Ratio (LR) for reporting the statistical weight of DNA inclusions. The LR presented in court is represented as:

$$LR = \frac{\Pr(E|H_1, I)}{\Pr(E|H_2, I)}$$

where  $E$  is the evidence,  $I$  is relevant case information,  $H_1$  is a proposition that represents one view point, and  $H_2$  is an alternate proposition that is mutually exclusive to the  $H_1$  proposition. This equation describes the ratio of the probabilities of the evidence given two competing propositions. Note that it is NOT the ratio of the probabilities of the propositions.

This LR is one term found in the odds form of Bayes' Theorem

$$\frac{\Pr(H_1|E, I)}{\Pr(H_2|E, I)} = \frac{\Pr(E|H_1, I)}{\Pr(E|H_2, I)} \times \frac{\Pr(H_1|I)}{\Pr(H_2|I)}$$

Posterior odds
LR
Prior odds

where posterior odds are the product of an LR and prior odds. Prior odds are the belief in the propositions based on other (non-LR) evidence. Posterior odds gives the final ratio of the propositions considering both an LR and the other evidence in the case. Note that it is posterior odds that address the ratio of the propositions after considering the evidence.

Recently there have been several court decisions and publications that address the use of Bayes' Theorem in the United States legal system in a negative light. Much of the criticism is directed toward a jury of lay persons being responsible for combining the prior odds with the LR presented by an expert to come to a final decision. There have been other decisions that seem to directly support the use of Bayesian thinking at court—although not by name. However, there are several aspects of the trial process where the court seems to both expect and instruct the jury to use a Bayesian framework—although not by that name.

Things get more complicated when the Hierarchy of Propositions is not followed, resulting in an LR for the DNA profile (subsource level) being transposed to the probability of guilt (offense level). Several of the negative publications and decisions seem to mix levels of this hierarchy as well as transpose an LR to the posterior odds.

This presentation will cover a review of the Hierarchy of Propositions, the three components of Bayes' Theorem, then look at current Federal Juror Instructions to see if there really is a discordance between Bayes' Theorem, forensic science, and the role of the decision maker (jury). The goal of this presentation is to encourage all stake holders in the justice system to start a dialogue with each other to better understand how each role relates to the other, how to work together to best inform the decision maker, and perhaps to discover some relationships that shows we might agree more than we realize. The goal of this presentation is to advance a dialogue between the scientist and both prosecuting and defense attorneys in how to best communicate DNA findings to a jury.

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### Forensic DNA, Testimony, Bayes' Theorem

## F27 A Critical Review of Admissibility of Canine Alerts in Arson Cases

Terry-Dawn Hewitt, LL.M.\*, McKenna Hewitt, Denver, CO 80224-3703

**Learning Overview:** After attending this presentation, attendees will be aware of the vast array of issues concerning Ignitable Liquid (IGL) detection canine alerts that should be considered by attorneys and fire investigation experts where one party proposes to proffer such alerts as evidence in criminal or civil arson cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by summarizing the surprising results from a comprehensive review of more than 100 American and Canadian court decisions mentioning IGL canine alerts, together with relevant scientific publications.

The National Fire Protection Association (NFPA) 921 Guide to Fire and Explosion Investigations is a consensus-based industry standard widely recognized as representing the standard of care for fire investigators.<sup>1</sup> In 1996, NFPA 921 added a section on Canine Teams.<sup>2</sup> The section was short, but made three important points: (1) the proper use of canines is to assist with the location and selection of samples for submission to a laboratory to be tested for the presence of IGLs; (2) the canine's nose may be more sensitive than laboratory testing, but a canine may not be able to distinguish between an IGL and background materials; and, (3) "Any canine alert not confirmed by laboratory analysis should not be considered validated." On its face, these points would seem relevant to a court's determination of whether evidence about IGL canine alerts is properly admissible in evidence as substantive evidence of the presence of an accelerant. Accelerants by definition include IGLs intentionally introduced to start a fire or speed its growth, so evidence of their presence is usually highly relevant to the proof that a fire is incendiary. Although changes have been made to the canine section over time, NFPA 921 has contained these points in every edition since 1996. Notwithstanding these cautions, courts in the United States have regularly admitted evidence of IGL canine alerts, even when the presence of IGLs is not confirmed by laboratory analysis.

Examining the court decisions (mostly in criminal cases) that mention IGL canine alerts as part of the evidence given at trial, this study made some remarkable findings. Here are a few examples. Often criminal defense attorneys failed to object to the admissibility of canine alerts or made no motion to require a reliability hearing under Daubert or the state rule determining admissibility of expert evidence. Where objections were raised to such evidence, many cases held that the alerts (even if unconfirmed by laboratory analysis) were admissible if corroborated by other evidence. Frequently, the corroborating evidence was the opinion of a fire investigator who found "pour patterns" and interpreted them to indicate the presence of accelerants in the absence of laboratory confirmation. While NFPA 921 has developed over time in terms of fire pattern interpretation, it is quite clear in the 2021 edition that such irregularly shaped fire patterns may have a number of explanations. Therefore, the presence of an IGL should be confirmed by laboratory testing and not be based solely on the interpretation of a fire pattern.

Perhaps what is most surprising is how many cases make no reference to NFPA 921 or other scientific literature that question the reliability of unconfirmed IGL canine alerts as evidence of the presence of an accelerant. Notwithstanding this body of cases where the majority admit evidence of IGL canine alerts, the consensus of the scientific community continues to grow stronger. The latest edition of NFPA 921 has published a substantially revised section on IGL canines. Most notably, NFPA 921 now says, "Any canine alert not confirmed by laboratory analysis should not be considered validated and, accordingly, should not be offered as direct or circumstantial evidence of the presence of an ignitable liquid in a criminal or civil case."<sup>3</sup> Hopefully this new consensus of the scientific community will have an impact on the use of IGL canine alerts in litigation.

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2. Tech. Committee on Fire Investigations. *Tentative Interim Amendment to NFPA 921 Guide for Fire and Explosion Investigations*. Quincy, MA: Natl Fire Protection Assn, 1996.
3. *NFPA 921*, 2021 ed., s. 17.7.7.

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### Canines, Fire Investigation, NFPA 921



## F28 Jury Trials During the COVID-19 Pandemic: Lessons Learned From Texas

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**Learning Overview:** After attending this presentation, attendees will understand Texas' approach to addressing challenges faced by the court system during the COVID-19 pandemic, including special considerations regarding remote expert testimony and efforts to keep testifying experts safe.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating one state's response plan to facilitating the effective ongoing administration of justice during the COVID-19 pandemic.

Texas' constitutional bill of rights provides that "the right of trial by jury shall remain inviolate." As many Texans were celebrating the dawn of a new decade on December 31, 2019, the original cluster of cases of the COVID-19 disease ("COVID-19") was identified in China. Less than three weeks later, the first diagnosed case of COVID-19 was identified in the United States. While Texas would not see its first diagnosed case of COVID-19 until March 4, 2020, the Texas judiciary began its involvement with the Governor's COVID-19 response in February. Based upon the information gathered during early briefings, the Texas judiciary activated its response plan, which has ultimately incorporated both limited in-person jury trials and the extensive use of online technology.

Before the pandemic, Texas courts averaged 186 jury trials per week. Beginning in mid-March, jury trials were suspended by the Texas Supreme Court except for in limited cases. During this presentation, attendees will learn about efforts undertaken by the Texas Office of Court Administration (OCA) to facilitate the effective ongoing administration of justice. Specific attention will be paid to considerations regarding remote expert testimony as well as challenges involved in keeping testifying experts safe, especially those with pre-existing conditions that may make them especially susceptible to COVID-19.

**Remote Hearings:** The COVID-19 pandemic created a technological revolution in Texas courts. OCA's first step in facilitating remote proceedings was to evaluate various technology platforms. OCA ultimately determined that Zoom would be the best platform for the Texas judiciary. To test this determination, OCA requested 20 judges test the platform with remote hearings during the week of March 16–20. Almost 100 proceedings were conducted during that week, with judges providing positive feedback about the platform's utility for remote hearings. With this feedback, OCA procured enough licenses for every judge in Texas to have a license so the full features and security of the Zoom platform would be available to those judges. Texas judges quickly embraced the technology, with approximately 440,000 remote hearings in every case type and type of proceeding, including bench and jury trials, with 1.3 million participants lasting almost one million hours held in the six-month period.

On August 12, 2020, Texas successfully held the nation's first virtual criminal jury trial in a misdemeanor case involving a traffic violation. Over 20,000 people tuned in via YouTube® to watch the trial proceedings.

**In-Person Jury Trials:** On May 27, the Texas Supreme Court issued an order prohibiting courts from holding in-person jury proceedings, including jury selection or a jury trial, any time before August 1. In addition, the Court required OCA to coordinate with the Regional Presiding Judges and local administrative judges to assist trial courts in conducting a limited number of in-person jury proceedings. In the May 27 order, the Court required consent from all parties to proceed with the limited jury trials. The consent requirement was later removed in the Eighteenth Emergency Order on June 29. The Court required OCA to submit a report to the Court outlining its observations regarding those jury proceedings and making recommendations regarding procedures. In subsequent emergency orders, the Court further delayed jury proceedings (with certain exceptions) until October 1. Before reviewing any jury proceeding requests, OCA consulted with infectious disease experts at the Texas Department of State Health Services (DSHS). Those meetings resulted in several best practices and answered questions that greatly assisted OCA in its efforts. DSHS staff were available to OCA staff throughout the spring and summer to assist with providing advice on matters affecting public health. This includes reviewing the local COVID case data in each county several days before the trial is scheduled to begin and relaying any concerns about moving forward.

On August 28, 2020, OCA issued its report and recommendations to the Court on the successes and challenges in addressing the COVID-19 pandemic.

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### COVID-19, Courts, Expert Testimony



## F29 Examining the Foundational Validity of Firearm and Toolmark Identification and the Continued Admission of Flawed “Science” in Court: A Call for Increased Scrutiny

Kyla J. Wells, JD\*, *The Legal Aid Society, Bronx, NY 10451*

**Learning Overview:** This presentation will offer attendees a defense perspective on the virtually unquestioned admission in courts of firearm and toolmark identification—a subjective pattern-matching discipline that lacks an adequate empirical basis. After attending this presentation, attendees will understand the faulty assumptions that underpin the firearm and toolmark identification field, the danger of wrongful convictions presented by the admission of this flawed evidence in courts, and the reasoning of recent federal and state court decisions limiting the admissibility of testimony regarding firearms identification at trial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in an effort to promote greater scrutiny of the discipline. The goal of this presentation is to shift thinking about the firearm and toolmark identification field, not only within the defense community, but among prosecutors and law enforcement as well. A willingness by all parties in the criminal justice system to closely examine firearms identification will lead to the scientific research necessary to adequately demonstrate the foundational validity of the field and thereby prevent wrongful convictions stemming from the admission of this evidence.

**Hypothesis:** For decades, the prosecution has presented the testimony of firearms identification experts as “scientific” proof that the bullets or shell casings recovered from a crime scene were fired from a particular gun to the exclusion of all other guns. With unqualified certainty, experts have explained that the bullets found at the crime scene must have come from the defendant’s gun or that bullets found at different crimes were fired from the same weapon, implicating the same shooter.

Three recent Reports issued in 2008, 2009, and 2016, two by the research arm of the National Academy of Science, and one by the President’s Council of Advisors on Science and Technology (PCAST), reject the claim that firearms and toolmark examination is a valid and reliable science.<sup>1-3</sup> The most recent 2016 Report, following a review of more than 2,000 articles and presentations by members of the forensic community, states unequivocally: firearms examination “falls short of the scientific criteria for foundational validity.”<sup>4</sup> These three interdisciplinary Reports authored by three separate committees of nationally recognized scientists and professionals find that the “fundamental assumptions” underlying firearms examination have not been demonstrated; that the theory is “not a scientific theory;” that the method is “subjective;” and that there is “insufficient empirical evidence” establishing validity and estimating reliability. Despite these flaws, judges routinely admit firearms identification evidence, while prosecutors vigorously fight for its protection and expanded use in courts.

After the first hearing of its kind in New York State, the Bronx County Supreme Court refused to admit testimony from a firearms examiner that bullets found at the scene of a shooting came from a particular firearm.<sup>5</sup> Troubled by the lack of empirical data, standards, and established error-rates in the field, the Court ruled that firearm and toolmark identification was not generally accepted in the relevant scientific community. This decision, along with other recent federal court decisions, call into question the long-standing acceptance of firearms identification evidence in courts.<sup>6,7</sup> This presentation will discuss the slow progress the defense bar has made in chipping away at the unquestioned acceptance of this evidence in court, while examining the flawed methodology upon which the firearms identification field is based.

### Reference(s):

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2. National Research Council, *Committee on Identifying the Needs of the Forensic Science Community, Strengthening Forensic Science in the United States: A Path Forward*, (2009).
3. President’s Council of Advisors on Science and Technology, *Forensic Science in Criminal Courts: Ensuring Validity of Feature-Comparison Methods*, (Sept. 20, 2016).
4. PCAST Report, p. 11
5. *People v. A.M.*, 68 Misc.3d 899 (Sup. Ct. Bronx Co. 2020).
6. *United States v. Shipp*, 422 F.Supp.3d 762 (E.D.N.Y. 2020).
7. *United States v. Tibbs*, No. 2016-CF1-1943, 2019 WL 4359486 (D.C. Super. Sep. 5 2019).

### Toolmarks, Firearms, Identification

## F30 Don't Shoot the Messenger: Firearm and Toolmark Evidence Is Still Reliable

Raymond Valerio, JD\*, Queens District Attorney, Kew Gardens, NY 11415

**Learning Overview:** The goal of this presentation is to illustrate the reliability of firearm and toolmark analysis despite recent court rulings limiting or precluding such evidence.

**Impact on the Forensic Science Community:** This presentation will impact attendees by providing wider perspective on recent court rulings on firearm and toolmark analysis, define the relevant scientific community, and explain how additional research has supported traditional techniques.

On January 13, 2018, an off-duty police officer observed, from his apartment window, a man wearing a “72” jersey fire two shots in the air and then enter a parked Sports Utility Vehicle (SUV). The officer called 911, described the shooter, and stayed on the phone as he observed uniformed police officers respond within minutes. These officers asked the passengers in the SUV to exit. There was one male wearing a “72” jersey (defendant A) and two females. The officers recovered one firearm inside one of the female’s purses (defendant B) and two spent shell casings on the road directly next to the SUV. Detectives identified the recovered cartridge casings as having been fired from the recovered weapon. Additionally, DNA results from the trigger of the firearm matched defendant A.

For decades, prosecutors have presented firearm and toolmark evidence at trial. But it was not until *People v. A & B*, that this State court made the surprising decision to hold a *Frye* hearing on this forensic science discipline—a discipline that is in no way a novel science.<sup>1</sup> Nonetheless, the Court ruled that the People were precluded from presenting evidence that recovered shell casings and test fires were identified as having been discharged from the recovered firearm.

A close examination of the decision will reveal, per this study, the court misapplied the *Frye* test, unjustifiably credited or discredited experts, and improperly dismissed the validation studies and the laboratory’s methodologies.

In *People v. LeGrand*, the Court of Appeals explained the *Frye* standard.<sup>2</sup> The Court held that “the *Frye* test asks ‘whether the accepted techniques, when properly performed, generate results accepted as reliable within the scientific community generally.’ ” (*Parker v. Mobil Oil Corp.*, quoting *People v. Wesley*, see also *Marsh v. Smythe*).<sup>3-5</sup>

After several days of testimony, the relevant scientific community was defined by the People’s witnesses: trained firearm and toolmark examiners; the 200+ accredited labs across the world that conduct the same analysis as the instant laboratory; the Organization of Scientific Area Committees (OSAC) Firearm and Toolmark Subcommittee; the State Commission on Forensic Science; as well as many statisticians and other non-firearm practitioners listed in the submitted validation studies. Among this community, firearm and toolmark examination far exceeds general acceptance.

Instead of counting all scientists’ votes, the Court credited the two defense witnesses (a law school dean and a psychiatrist) as representing a completely unknown, yet statistically significant, group of non-practitioner scientists who do not support firearm and toolmark analysis.

The Court’s decision discounted peer-reviewed published validation studies that demonstrate the accuracy of firearm and toolmark examination, stringent adherence to the highest international accreditation standards, the laboratory’s “blinded” review procedures, and independent scientific research.

### Reference(s):

1. *People v. A & B*, 2020 WL 3564693 (Sup Ct, Bronx Cty, June 30, 2020).
2. *People v. LeGrand*, 8 N.Y. 3d 449 (2007).
3. *Parker v. Mobil Oil Corp.*, 7 N.Y.3d 434, 446 (2006).
4. *People v. Wesley*, 83 N.Y.2d 417, 422 (1994).
5. *Marsh v. Smythe*, 12 A.D.3d 307 (1<sup>st</sup> Dept. 2004).

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### Firearm and Toolmark, *Frye*, Relevant Scientific Community

## G1 Limousine Fire

*Roland G. Chew, DDS\*, Fremont, CA 94538*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of having and coordinating a mass disaster plan. When a disaster occurs, it may require multiple people be dentally identified. Having a disaster plan ready has been discussed many times. Disasters can be small or large, but the fundamental preparation is needed. On a large-scale disaster, we have the Disaster Mortuary Operational Response Team (DMORT) at the federal level to help with the recovery and identification. However, on a smaller scale such as a wildfire, multiple victims in a motor vehicle accident, or even a house fire, the local forensic dentist needs to be prepared to deal with multiple victims. The case presented will provide the fundamental preparations required to handle dental identifications in an orderly and systematic fashion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a reminder that multiple victims in an accident need to be processed in a systematic way to make sure all key information is obtained from antemortem records to postmortem records. As a result, an accurate identification may be made by minimizing any loss of information, inaccuracy, and/or confusion.

The case began on an evening in the spring of 2016; a couple had just been married. The bride and her bridal party were off to the hotel for the wedding reception. The limousine carrying the driver and nine passengers was traveling over the San Mateo Bridge in the San Francisco Bay Area when it caught fire. There was panic in the back cabin of the car but the driver was unaware it was because of a fire. He interpreted the noise as people celebrating a recent marriage. By the time the driver became aware of the fire in the back cabin of the limousine, the driver stopped in the middle of the bridge. Minutes later, the back cabin was engulfed in flames. The driver and four females were able to escape the fire, but the bride and four other occupants did not survive.

A call was received from the county sheriff's office. It was confirmed that records needed to be obtained and instructions were given as to what the adequate records were, such as X-rays with date, written treatment records, and/or a list of completed services.

The bodies were severely burned and not viewable for an open-casket funeral. Since this was a case involving multiple victims, accuracy of the bodies was critical so that the families could grieve for their loved ones and lay them to rest. In the morgue, help was divided into different groups with different responsibilities such as starting paperwork with case number and postmortem pictures, resecting jaws, X-rays of the teeth and jaws, postmortem charting, antemortem charting, and lastly, comparison for identification.

Attendees will understand that there needs to be an orderly process involved in the identification to be complete and thorough.

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### Postmortem, Points of Concordance, Positive Identification

## G2 Intra-Alveolar Photogrammetry Scanning of Empty Dental Sockets of Teeth Missing Postmortem for Root Morphology Analysis: A Case Study

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**Learning Overview:** After attending this presentation, attendees will have an understanding of how the identification process of human remains with teeth lost postmortem can still be performed by reconstructing the morphology of missing teeth roots using an intra-alveolar technique.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the results of the use of a photogrammetry tool used by dentists for prosthetics treatments and its application in the field of forensic dental identification.

Forensic odontologists may have to perform a dental autopsy of unidentified human remains with missing teeth lost postmortem. Root morphology and the shape of treated as well as sound teeth could offer identifying features that can be useful within the forensic dental identification process.<sup>1-3</sup> In order not to lose this data, several techniques have been proposed in order to obtain a replica of the empty alveolar socket of the skeletal remains and thus the morphology of the missing roots. Currently, intra-alveolar morphology of empty sockets may be assessed with Computed Tomography (CT) scanning, periapical and panoramic radiographs, and silicon or alginate impressions.<sup>4-6</sup> The present study aims to assess the intra-alveolar morphology in empty dental sockets using photogrammetry scanning to reconstruct the 3D shapes of the roots of teeth missing postmortem.<sup>7</sup> Photogrammetry has reached advances in the field of digital photography and combining with a specific software has made this tool reliable and accurate and a well-established resource in prosthetic dentistry.<sup>8</sup> In dentistry this device, similar to an intraoral camera, will eventually replace the existing procedures that involve taking impressions with impression materials (alginate or silicone), than making a casting.

A dry unidentified skull was used for this study. The upper jaw with empty dental sockets was scanned using an intraoral scanner. Roots were reconstructed digitally in three dimensions, then compared with the radiographic images. A set of ten records formed the database for the evaluation of empty dental sockets, and the digital models were rotated into the specific dental planes to allow the comparison.

This study is devoted to data recording using an intraoral scanner as an alternative impression tool but with the advantage of a contactless technique. It was determined that teeth missing postmortem do not necessarily invalidate the identification process.

Per this study, the use of photogrammetry through intraoral scanners is a preferable technique compared to any traditional impression technique as it preserves and protects human bone from any alterations. Also, an intraoral scanner using photogrammetry should become one of the auxiliary tools of a dental autopsy, as it is a portable device, allowing accurate recording in any scenario.

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### Missing Teeth, Photogrammetry, Human Identification

## G3 The Correlation Between Root Translucency, Calcium Hydroxyapatite Content, and DNA Preservation in Teeth for Unidentified Human Remains (UHR) Investigations

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**Learning Overview:** The goal of this presentation is to provide attendees with an understanding of tooth microstructure, an overview of the pattern of increased root translucency with chronological age, a summary of diagenetic processes that impact the integrity of a tooth's mineral matrix, and an assessment of the potential correlation between these variables and DNA preservation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that knowledge of tooth microstructure—and understanding changes that occur to the mineralized matrix over time—can guide scientists in making informed decisions regarding optimal sampling from highly decomposed or skeletonized human remains for forensic DNA testing.

Human teeth are composed of three hard tissue layers (enamel, dentin, and cementum) that surround an inner chamber filled with vascularized soft tissue (pulp). Although pulp (like most soft tissues) is subject to rapid decomposition postmortem, hard tissues are more resistant to environmental insults. The endogenous genetic material protected by a tooth's rigid, mineralized hard tissues is often targeted for forensic DNA testing in missing persons and UHR investigations. One particular component of teeth—the inorganic mineral matrix (calcium hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ )—is purported to play a major role in postmortem DNA preservation. Numerous research studies demonstrate that electrostatic interactions occur between positively charged calcium residues in hydroxyapatite and negatively charged phosphate groups in the DNA backbone. These interactions with the hydroxyapatite matrix are believed to protect DNA from damage. However, although hydroxyapatite is thermodynamically stable under antemortem physiological conditions, changes to this mineral matrix occur over time as a decedent's remains are exposed to destructive environmental factors such as heat, humidity, ultraviolet light, microbial attack, and acidic soil. Alterations to the hydroxyapatite mineral matrix occur during a process called diagenesis and can involve substitution of calcium ions ( $\text{Ca}^{2+}$ ) with carbonate ( $\text{CO}_3^{2-}$ ) residues. When this occurs, DNA molecules dissociate from the mineral matrix and become more susceptible to damage.

The goal of this research is to determine if an established, validated forensic odontological method of age estimation for decomposed or skeletonized human remains (i.e., evaluation of tooth root translucency) can also be a reliable predictive parameter for downstream DNA typing success. Approximately 70% of dentin in the roots of human teeth is composed of hydroxyapatite crystals, and it has been asserted that there is a direct relationship between increased tooth root translucency and decalcification of hydroxyapatite. There may also be a direct relationship between tooth root translucency and DNA preservation within a tooth's microstructure. This research involved: (1) microscopic examination, measurement, and photo-documentation of root dentin translucency in intact and sectioned teeth ( $n=54$ ) from human subjects of known chronological age; (2) X-ray Diffraction (XRD) of pulverized tooth powder to assess the degree of carbonate substitution within the calcium hydroxyapatite matrix of each tooth; and (3) DNA extraction from tooth matrices to assess the correlation between degree of root dentin translucency and the quantity/quality of the DNA recovered.

The Crystallinity Index (CI) of each tooth was calculated using XRD data. Analyses for this sample set ( $n=54$ ) indicate that the CI decreases as the existence of carbonated (calcium-deficient) hydroxyapatite increases; similarly, a general trend of lower CI values were observed for teeth exhibiting greater degrees of root translucency and with increasing chronological age of the donor. Research is ongoing to generate additional data to determine if a direct correlation indeed exists between higher levels of carbonated (calcium-deficient) hydroxyapatite and decreased DNA recovery. Although weight-bearing long bones (femora, tibiae) and molar teeth are the current preferred skeletal sample types for forensic DNA testing (due to the protection afforded by the compactness and rigidity of their macrostructure), changes to a bone's or tooth's microstructure should also be considered in the development of Standard Operating Procedures (SOPs) or "best practices" models for human remains identification. Molecular changes to tooth microstructure due to the chronological aging process and postmortem environmental exposure—and the correlation between these changes to the quantity/quality of recoverable endogenous DNA—has not yet been the subject of investigation. Empirical data from this study could provide the framework to develop general guidelines for forensic odontologists in the field to use in evaluating a tooth's potential for DNA profiling success based on root translucency.

### Tooth Root Translucency, Hydroxyapatite, Unidentified Human Remains

## G4 Cementum Annuli: Technique, Microscopy, and Age Estimation

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**Learning Overview:** The goals of this presentation are: (1) to make attendees aware of the importance of tooth cementum annuli; (2) to increase knowledge of the procedure of analysis for Trichloroacetic Acid (TCA); and (3) to demonstrate the significant correlation between the annuli count, predicted age, and actual chronologic age.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees understand the value of age estimation using histology of cementum annuli enumeration on select root regions.

Mineralized dental tissues possess superb postmortem longevity. While cementum annuli enumeration studies of modern and zooarchaeological wild deer, bear, and wolf are well documented as an accurate age indicator, application to human teeth has been histologically and methodologically far less successful. Recent research indicates that cementum annuli may be a more reliable age indicator than other parts of the skeleton.

This study describes the thin section procedure and enumeration of cementum on all tooth types and root locations using dissecting, light, and Scanning Electron Microscopy (SEM). Deciduous and permanent teeth ( $n=108$ ) extracted from general dental and oral surgical practices because of periodontal disease and orthodontic and prosthetic reasons were used with known age, sex, and ancestry. Teeth were epoxy 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. The dissecting microscope at 5X–10X assessed cementum quality, thickness, and dentin coverage. At higher magnification (100X–200X), the SEM documented the structural reality of annuli and the Cementodentinal Junction (CDJ). The light microscope at 25X–50X allowed annuli counting for age estimation. A second sample was prepared using black bear, red fox, and macaque teeth, and a third sample was prepared using historic teeth. The second and third samples were exposed to “real” seasonal temperature fluctuations.

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 damaged or thinned many times from periodontal events and the thickened, cellular apical region reveals a more “confusing” and unassignable array of annuli. Countable 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 post-eruption for each tooth was added to the annuli count, found as a portion of the countable, measurable bands against the total measured cementum thickness. Results helped estimate 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 required to determine environmental effects such as temperature on cementum annuli formation.

### Cementum Annuli, Dental Age Estimation, Mineralized Tissue Histology

## G5 Obstructive Sleep Apnea-Hypopnea Syndrome (OSAHS): Medicolegal Implications and the Role of the Dentist in a Multidisciplinary Approach

Francesca Zangari, MD\*, Ravenna 48121, ITALY

**Learning Overview:** By attending this presentation, attendees will better understand the medicolegal aspects correlated to OSAHS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of a multidisciplinary approach wherein the dentist can potentially play an important diagnostic and therapeutic role.

OSAHS is a common sleep breathing disorder characterized by disruptive snoring and repetitive upper airway complete (apnoea) or partial (hypopnoea) collapse with increased airflow resistance during sleep, resulting in oxygen desaturation and sleep fragmentation.<sup>1-3</sup> Repetitive episodes of intermittent hypoxia are responsible for pathophysiological consequences that increased morbidity and mortality linked to cardiovascular, cerebrovascular and metabolic diseases.<sup>4</sup> OSAHS has been estimated to occur in around 24% of middle-aged men and 9% of women, affecting 4% of men and 2% of women in the middle-aged workforce, resulting in high costs and lost workdays.<sup>5-7</sup> It has been also found that among adults aged 30–69 years, 17% of adults had mild or worse sleep disordered breathing, and 5.7% of adults had moderate or worse sleep disordered breathing.<sup>8</sup>

Owing to its prevalence, OSAHS is recognized as a significant public health issue, which can manifest serious physical and social consequences if not managed properly. Medical costs can be significantly reduced when effective diagnosis and treatment are performed early.<sup>9-11</sup> This disorder, apart from potentially leading to an impaired quality of life for its signs and symptoms (excessive daytime sleepiness, irritability, impaired cognitive ability, and deficits in the domains of attention/vigilance), has been also associated with a high risk for motor vehicle accidents.<sup>12,13</sup> Drivers with OSAHS have roughly twice the risk of crashing as compared to healthy drivers. Physicians have a responsibility to promptly detect medical conditions that may impair driving and cause an increased risk of harm to the patient or the public.<sup>14-16</sup> So, a patient involved in a drowsy driving accident resulting in serious injury or death is the most likely situation in which physicians could face charges of legal negligence in relation to management of a patient with OSAHS.<sup>17,18</sup> However, state regulations for physician reporting of patients with medical conditions that may render them unfit for driving safely vary from “no requirement” to mandatory reporting of all patients with a diagnosis listed as reportable. Physicians are also in a very difficult position if they have to counsel their patients on the best attitude when traveling by car. The situation is even worse for commercial drivers for whom the legislation is more severe but leaves sleep apnea uncovered in many countries.<sup>19</sup> This is all the more disturbing if one considers that sleep apnea can be suspected, screened, and diagnosed with relative ease, and that once diagnosed the adequate treatment allows for safe driving.

In recent decades, dentists have become increasingly involved in the treatment of disorders that also fall within the domain of other medical specialists, OSAHS included. The awareness of having a potential diagnostic and therapeutic role stems from a growing recognition of orofacial characteristics as important developmental factors and from the realization that they have therapeutic implications.<sup>20</sup> These trends indicate the need for optimal collaboration among the different specialists involved. From the screening perspective, dentists, because of their contact with many members of the general population during routine examinations, are ideally placed to screen for potential OSAHS sufferers. They can recognize patients with suspected OSA through the identification of anatomic risk factors or symptoms, administer appropriate screening questionnaires, and refer patients at risk of OSA to sleep medicine physicians. As regards treatment, an increasing body of published literature reflects the growing worldwide recognition that oral devices have a role to play in the treatment of OSAHS. Standard treatment with Continuous Positive Airway Pressure (CPAP) is highly efficacious for OSAHS, but adherence to the treatment limits its overall effectiveness.<sup>21</sup> Oral appliance therapy, which aims at enlarging the upper airway during sleep by holding the mandible in a forward and downward position, can be a viable alternative in the treatment of OSAHS, especially in the mild and moderate cases and in patients unwilling or unable to tolerate CPAP.<sup>22-24</sup>

This presentation discusses the most relevant medical-legal aspects correlated to OSAHS and highlights the importance of a multidisciplinary approach wherein the dentist plays a significant prevention, diagnostic, and therapeutic role.

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## Obstructive Sleep Apnea, Traffic Accidents, Dental Sleep Medicine



## G6 Computed Tomography (CT) -Based Dental Identification—Changing the Paradigm and Practice of Dental Identification

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**Learning Overview:** After attending this presentation, attendees will understand how the harvesting of anatomically important data from medical CT images were used with Cone Beam CT (CBCT) software to aid in dental identification of a small multiple-fatality incident.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining the methodology to create postmortem images suitable for the efficient identification of deceased individuals and eliminate biological/body substance or radiation exposure to the operator.

Comparison of postmortem dental findings to antemortem dental records is a well-established, frequently used scientific means of human identification. Its initial use is deeply rooted in history dating back to the Battle of Bunker Hill during the American Revolution where the first dental identification of a soldier was made by a dentist and has been widely used since as a well-accepted means of establishing identity.<sup>1</sup> Present-day dental identification relies heavily on the comparison of antemortem and postmortem dental records and most frequently involves the comparison of both antemortem and postmortem dental radiographs because they are objective data.

Many medical examiner facilities either have dedicated CT units or access to conventional CT scanners that are used as part of the normal hospital practice. The mathematics behind CT radiography was developed in 1917 by Joseph Radon. Its application was adapted by Sir Godfrey Hounsfield—whose surname is immortalized as a measure of density—for clinical use in commercial CT scanners since 1971.<sup>2,3</sup> Dentistry has adapted a form of CT scanning that uses a cone-shaped beam and is thus termed CBCT. CBCT is presently being used in many aspects of dentistry, including osseointegrated implant planning, orthodontics, endodontics, investigation of pathology, and assessment prior to complex dental extractions.<sup>3</sup>

All modern CT scanners store their files in either a proprietary software or, for portability sake, in a universally readable format known as Digital Imaging and Communications (DICOM). DICOM images from mortuary medical CT scanners can be imported, read, and manipulated in third-party image processing and viewing software found in oral and maxillofacial radiology practices and elsewhere. This presentation will discuss the means by which the harvesting of anatomically important data from medical CTs and conversion of these DICOM files from medical CT scans can be formatted to provide crisp, clear postmortem dental images for ulterior forensic comparison. This was first done in the context of a small multiple fatality incident involving seven passengers in a small plane crash in Ontario, Canada. Medical CT scans for each of the severely damaged victims in this incident were acquired without needing to open the sealed body bag. The maxillofacial DICOM files from the medical CT scanner were manipulated with commercially available CBCT software used in dental applications to provide postmortem pantomographic-type images and compared to available antemortem records. A collaborative effort of experts allowed expeditious identification of all seven individuals killed in a small airplane crash.

This novel adaptation of existing technology can be performed very quickly and avoids any biological/body substance or radiation exposure to the operator. It requires no dedicated intraoral radiation sensors nor conventional films and processing chemistry. There is no need for the pathology assistant, medical imaging technologists, or forensic odontologist to touch the body. Further, there is no need to alter the position of the body as this can be corrected in the program. Images can be manipulated with respect to position, contrast, density, volumes examined, and can be exported to other programs such as Adobe® Photoshop® for further refinement. The original data set of DICOM images is maintained and the steps in the process can be archived for the sake of continuity of evidence. It is this study's contention that this technology is a boon to both single body and multiple fatality situations where postmortem images can be rapidly acquired and processed and in situations where single or multiple comparisons need to be made.

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### Forensic Odontology, Human Identification, Cone Beam Computed Tomography

## **G7 When There Are No Typical Dental Remains, “Thinking Outside the Bag” Is Required to Make a Positive Dental Identification**

*Randolph L. Mitchell, DMD\*, Lyons, NY 14489*

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**Learning Overview:** After attending this presentation, attendees will recognize and understand the need for a thorough examination of the remains in order to locate all dental remains that could possibly be useful in making a positive dental identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining why sometimes pertinent dental remains may not seem to be useful compared to the typical dental remains used in making dental identifications and why it is important to be thorough in the search for all possible exemplars.

Dental identifications are all unique puzzles that we endeavor to solve scientifically. Some of the puzzles are incredibly basic and easy to solve. Others seem to be overwhelming or nearly impossible to solve upon initial examination of the remains, either due to the condition of the remains or a seeming lack of definitive exemplars. These are the cases that are the most challenging, requiring evaluation of the case from a unique perspective based on the information that is available. This presentation will use an actual case study to show how the importance of locating and examining all of the available dental structures, the charting and cataloging of these remains, the importance of using sound logic, and using basic dental anatomy learned long ago in dental school can help to yield a scientific positive dental identification by comparison to antemortem records.

The fire in this case was intense, burning long and hard, resulting in severe damage to the dental structures and the rest of the body. The clinical crowns of many of the teeth were calcined to the point where they were essentially ashes and crumbled upon contact with any dental instrument. The mandible and maxilla were burned to such an extent that much of the cortical bone was gone, leaving a bas relief model of the shape of the roots and trabecular bone with no real clinical crown structure present to be evaluated. Many individual roots were present in “loose form,” meaning that they were mixed in with the remains in the body bag and had to be “sifted through” in order to be found, though some roots were still present in the mandibular and maxillary structures, again as roots with no crowns present. This identification was accomplished using slow and careful examination of root structure, unique root morphology, and ultimately enhanced by the presence of a “unique surprise perfect exemplar,” missed among the charred fragments in the early stages of evaluating the dental structures present.

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### **Examination, Thoroughness, Logic**



## G8 Ghost Ship Fire—Up in Smoke

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**Learning Overview:** After attending this presentation, attendees will understand what occurred at the Ghost Ship Fire, what was involved in body recovery, and how bodies were identified. Case examples will be examined.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a reminder to be prepared for any size disaster and by informing attendees how to manage multiple body identifications by dental means. This presentation will emphasize the need for dental identification as a means of accurate identification as well as an expedient form of identification. Many times, prior to going to the morgue in a fire case, one is concerned with the quality of the body (teeth) to be examined. Questions in this fire case were how long were the bodies exposed to the fire, did the soft tissue protect the integrity of the teeth, were teeth damaged during the recovery process, are the burned teeth going to be salvageable for evaluation and comparison, and are the antemortem records and postmortem records adequate for comparison.

Dental identification is based upon multiple points of concordance of records being compared. It may involve tooth anatomy such as clinical crown, root size, and formation. Identification also relies on tooth position and restorations placed or not placed on the tooth. There are many anatomical landmarks that may be evaluated and compared to in aiding to make a positive, negative, or inconclusive identification. In order to do a postmortem dental evaluation and identification, antemortem records must be obtained.

Three cases are presented here to review how victims were identified in this fatal fire.

**Case 1:** This person had alloy restorations on the buccal of the second molars. They are similar, but they can also be found on many other people. Through further investigation, indirect crowns have unique features as the dental lab and dentist will have unique preparations and designs during the fabrication process of the crowns. These allowed identification of this person more conclusively through these unique features.

**Case 2:** This person had tooth restorations on multiple teeth that were unique in size and shape and allowed for a conclusive determination of identification.

**Case 3:** In this case, as well as each case in this fire, the cause of death was smoke inhalation. The bodies were not severely burned and the teeth were well preserved. This case demonstrates the usefulness of restorations and unique dental anatomy for identification.

This catastrophic fire and cases presented will allow the forensic odontologist to appreciate the importance of attaining good antemortem records in case they need to be compared to the postmortem records. This will allow the odontologist to make a statement of identification in a timely matter.

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### Dental Identification, Burned Victims, Tooth Anatomy

## G9 Selecting the Skeletal Mandibular Identifiers With the Strongest Potential for Human Identification on Panoramic Radiographs

Anca R. Iliescu, DMD\*, Leuven 3000, BELGIUM; Cezar Capitaneanu, DMD, PhD, KU Leuven, Leuven B3000, BELGIUM; Debora Hürter, DMD, Private Dental Practice, Wuppertal 42117, GERMANY; Steffen Fieuws, Leuven, VB, BELGIUM; Jannick De Tobel, PhD, KU Leuven, Imaging and Pathology - Forensic Odontology, Leuven, Vlaams-Brabant 3000, BELGIUM; Patrick W. Thevissen, PhD, KU Leuven BE 419052173, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand that the mandible proves to be valuable for identification through its skeletal morphometric identifiers, and attendees will also learn how to implement the proposed method in forensic casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the basis for an alternative identification method based on skeletal mandibular traits.

**Background:** The gold standard of odontological identification relies on dental treatment, morphology, and morphometry comparisons between antemortem and postmortem dental files.<sup>1</sup> However, the decrease in dental restorations reduces the occurrence of unique dental identifiers.<sup>2</sup> Nevertheless, dental radiographs contain more than just dental information. For instance, panoramic radiographs depict the entire mandible. Therefore, the current study aimed to select the best mandibular morphological traits with a strong identifying capacity.

**Material and Methods:** One hundred eighty-five digital panoramic radiographs (94 males and 91 females; age range 16–66 years) were retrospectively collected from a private dental clinic in Brussels, Belgium, and imported into image enhancement software. Four landmarks were located on the mandible, namely the most superior point of the Condyle Right/Left (CONR/L), the most superior point of the Coronoid Right/Left (CORR/L), the most superior point of the mandibular Lingula Right/Left (LINR/L), and the most mesial point of the Mental Foramen Right/Left (MMFR/L). Based on the landmarks, five linear measurements were performed bilaterally. Using the linear measurements, six angles and ten ratios were calculated.

In order to determine and quantify the identifying capacity, three groups of statistics were considered: (1) inter-observer agreement quantified by Intra-Class Correlation (ICC) and Within-Subject Coefficient of Variation (WSCV); (2) mean “potential set,” which represents the percentage of subjects in the antemortem reference dataset one at least needs to consider in order to detect the target (i.e., the unknown subject); and (3) Spearman correlation between parameters.<sup>3</sup> Based on those statistics, a selection of parameters was conducted to establish a three-step univariate cascade (high caseload [e.g., mass disaster]) as well as a multivariate cascade (low caseload). The cascades provide the user with an easy and practical application of the selected parameters, with the benefit of further narrowing the antemortem dataset as the user progresses through the steps of the cascade.

**Results:** In the univariate setting, the following parameters proved to have the best identifying capacity: ratio 3 on the right side (between lines CONR–CORR and LINR–MMFR) with ICC 0.90, WSCV 4.8%, and mean potential set 13%; ratio 4 (between lines CONR/L–CORR/L and MMFR–MMFL) with ICC 0.92, WSCV 8.9%, and mean potential set 13%; and angle 4 on the left side (between landmarks LINL, MMFL, and MMFR) with ICC 0.91, WSCV 1.2%, and mean potential set of 18%. The correlation coefficients between angle 4 and ratios 3 and 4 ranged from 0.01 to 0.33, indicating that they provide complementarity rather than overlapping identifying information. Combining parameters in the multivariate setting, the identifying capacity improved drastically: all ratios combined (mean potential set 1.3%) and all angles combined (mean potential set 2.6%).

**Conclusions:** Specific univariate and multivariate cascades were provided to select the parameters with the best identifying capacity, based on morphometric mandibular traits. In high caseload assignments, a single ratio or a single angle already narrows the set of potential matches, but the mean potential set remains relatively large. Combining all ratios or all angles drastically increases the certainty of the match, and is therefore recommended, especially in low caseload assignments.

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### Forensic Odontology, Human Identification, Mandible

## G10 A Fractal Analysis of Bone Trabeculae—Artificial Intelligence in Identification

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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, 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 previous research projects, *The Trabecular Bone in Identification*, *The Trabecular Bone in Identification—Algorithms and Fractal Analysis*, *Fractalyse Software—The Analysis of the Trabecular Bone in Identification*, and *Fractalyse Software—The Analysis of the Trabecular Bone in Identification (The Continuation)*, the current research deals with the concept of artificial intelligence in the morphometric analysis and comparison of trabecular bone patterns.<sup>12-16</sup> Outcomes suggest that the development of a new software with a revolutionary approach to handling human trabecular jaws' 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, Fractal Analysis, Artificial Intelligence

## G11 Selecting the Dental Morphological Identifiers With the Strongest Potential for Human Identification on Panoramic Radiographs

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**Learning Overview:** After attending this presentation, attendees will understand how dental morphological features on panoramic radiographs can be used effectively for human identification by applying a three-step cascade (univariate and multivariate) with the strongest identifying capacity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying the capacity of dental morphological features on panoramic radiographs for identification and by providing a three-step cascade that can be implemented immediately in high (univariate) or low (multivariate) caseload assignments.

**Background:** Dental identification relies on comparing dental treatment features, odontogenic morphological features, or a combination of both. Treatment features are usually the first line of assessment used.<sup>1,2</sup> However, factors such as incomplete dental records and the ongoing decline in the number of dental restorations have increased the importance of morphological features.<sup>3-5</sup> Unfortunately, to date, there is no conclusive evidence that the human dentition is indeed unique, which is the basis in being able to use morphological dental features for identification. Therefore, the current study aimed to select the best dental morphological identifiers for human identification.<sup>6</sup>

**Material and Methods:** Sixty-two digital panoramic radiographs from 31 females and 31 males (age ranged from 13 to 56 years old) were collected retrospectively from a private dental clinic in Belgium. Since the mandibular teeth are often more clearly depicted on panoramic radiographs than the maxillary teeth, the focus of this pilot study was on all seven mandibular left permanent teeth (excluding third molars), in which six measurements were performed: Tooth Length (TL), Crown Length (CL), Root Length (RL), Crown Width (CW), Cervical Width (CEJW), and Root Width (RW). Nine length-width ratios were then calculated for each tooth, using these measurements. To determine and quantify the identifying capacity, three groups of statistics were considered: (1) inter-observer agreement quantified by Intra-Class Correlation (ICC) and Within-Subject Coefficient of Variation (WSCV); (2) mean “potential set,” which represents the percentage of subjects in the antemortem reference dataset one needs to at least consider in order to detect the target (i.e., the unknown subject in the antemortem reference dataset); and (3) Spearman correlation between parameters. Based on those statistics, a selection of parameters was conducted to establish a step-by-step approach that can be put into practice immediately. Forensic odontologists can choose a cascade of steps to narrow potential matches (high caseload [e.g., mass disaster, univariate plan]) or choose one step to be applied to their specific case (low caseload [e.g., domestic fire, multivariate plan]).

**Results:** In the univariate setting, the following parameters proved to have the best identifying capacity: TL/CW for tooth 36 (ICC 0.82; WSCV 5.1; mean potential set 14%), TL/CEJW for tooth 35 (ICC 0.87; WSCV 3.9; mean potential set 15%), and TL/RW for tooth 32 (ICC 0.89; WSCV 4.2; mean potential set 16%). The correlations between these three parameters ranged from 0.24 to 0.47, indicating they provide complementary rather than overlapping identifying information. Compared to single parameters, combining parameters substantially improved the identifying capacity. In the multivariate setting, the following parameters proved to have the best identifying capacity: all parameters combined for tooth 31 (mean potential set 8%), for tooth 35 (mean potential set 12%), and for tooth 32 (mean potential set 16%).

**Conclusion:** Three-step cascades were provided to select the parameters with the best identifying capacity in mandibular permanent teeth. In high caseload assignments, a single parameter in a specific tooth narrows the set of potential matches, but the mean potential set remains relatively large. In low caseload assignments, it is recommended to combine all parameters of a specific tooth to increase the certainty of the match. In particular, tooth 31 proved to be the strongest identifier.

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### Forensic Odontology, Human Identification, Tooth Dimensions

## G12 The Identification of Fiery Highway Crash Remains

*Robert C. Walcott, DDS\*, Moraga, CA 94556*

**Learning Overview:** After attending this presentation, attendees will understand key forensic odontology techniques to identify severely burnt remains with only three anterior antemortem periapical radiographs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating key investigatory techniques to identify charred remains. Specific instruments used will be discussed. The California Highway Patrol (CHP), Contra Costa County Sheriff, news reports, and forensic odontology all contributed to the identification.

The CHP reported on September 29, 2019, at 4:00 a.m. four cars were traveling about 65mph northbound on Interstate 80 in Richmond, CA. The vehicles were actually involved in a series of at least three different crashes. The vehicles in order were a Nissan® Altima®, a Honda® CRV®, a Honda® Civic®, and a Hyundai® Sonata®. The driver of the Altima® and an adult passenger hit the CRV® and the center concrete wall divider, resulting in the passenger's death. The impact to the CRV® caused self-ignition resulting in temperatures up to 495°F and the driver's death. The CRV® also hit the Civic®, which collided with the Sonata®. All other drivers sustained serious injuries and were transported to Highland hospital. The drivers of all vehicles were identified by CHP except the Honda® CRV® driver. This driver sustained fourth-degree burns penetrating skin and muscle, fifth-degree burns penetrating to bone, and sixth-degree burns that charred bone. No immediate physical recognition was possible.

The remains were transported to the Contra Costa County Coroner's office for documenting, weighing, measuring, tagging, and storing. This office requested a forensic odontology examination. There were only three antemortem periapical films, two of tooth #24 dated 09/23/2015 and one of tooth #7 dated 10/21/2015. The maxilla and mandible were resected and cleaned. The instruments utilized were Stihl® bypass shears, eight-inch tissue retractors, toothed forceps, curved and straight hemostats, and an eight-inch Rochester® curved heavy forceps. A scalpel, #8P handle with a #22 blade, was also used. Photographs were taken and the dentition was charted on the Contra Costa County-Odontology report. A digital radiograph survey was completed utilizing Dexis® software with a Nomad® hand-held portable unit. The examination revealed that there were two teeth missing antemortem, the maxillary right third molar #1 and right lateral incisor #7. There was an implant present for the lateral incisor; however, no crown was in place. There was a removable stay plate that replaced tooth #7. Tooth #24 had a root canal and composite. The left sides of the maxilla and mandible had sustained sixth-degree burns. Seven molars had occlusal composites (3, 14, 15, 18, 19, 30, and 31). Due to the impact, teeth 8, 9, 10, 11, 12, 13, and 14 were fractured. Teeth 10 and 11 were fractured off at the alveolar crest as were teeth 18, 20, 21, 22, and 23.

After comparing the antemortem radiographs with the postmortem full-mouth films, a positive identification was achieved and reported to the Contra Costa Sheriff-Coroner. On October 1, 2019, Mario Martinez was identified.

This case study demonstrates the application of several key techniques to identify severe charred remains.

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### Burnt, Techniques, Identification



## G13 The Application of an Intraoral Scanner to Identify Monozygotic (MZ) Twins

*Botond Simon, DMD\*, Budapest 1035, HUNGARY; Janos Vag, Semmelweis University, Budapest, HUNGARY*

**Learning Overview:** After attending this presentation, attendees will have learned of the possible usage of an intraoral scanner for human identification based on palatal morphology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing attendees to a quick, easy-to-use, affordable method for forensic identification with an intraoral scanner. If among the victims there are MZ twins, this method will be able to help the forensic odontologist identify them.

**Background:** DNA base identification is a proper and high specificity method.<sup>1</sup> However, identification could be challenged in a situation where there is no database or the DNA sequence is almost identical, as in the case of MZ twins.<sup>2</sup> The aim of this study was to introduce a novel forensic method for distinguishing between almost identical MZ twins by means of an intraoral scanner using the 3D digital pattern of the human palate.

**Methods:** The palatal area of 64 MZ twins and 33 same-sex Dizygotic (DZ) twins (DZSS) and seven Opposite-Sex Dizygotic twins (DZOS) were scanned three times with an intraoral scanner. From the scanned data, a Stereolithography (STL) file was created and exported into the GOM Inspect<sup>®</sup> inspection software. All scans within a twin pair were superimposed on each other. The average deviation between scans of the same subject (Intra-Subject Deviation [ISD]) and between scans of the two siblings within a twin pair (Intra-Twin Deviation [ITD]) was measured. One-sided tolerance interval covering 99% of the population with 99% confidence was calculated for the ISD (upper limit) and the ITD (lower limit).

**Results:** The mean ISD of the palatal scan was  $35.3\mu\text{m} \pm 0.78\mu\text{m}$ . The calculated upper tolerance limit was  $95\mu\text{m}$ . The mean ITD of MZ twins ( $406\mu\text{m} \pm 15\mu\text{m}$ ) was significantly ( $p < 0.001$ ) higher than the ISD, and it was significantly lower than the ITD of DZSS twins ( $594\mu\text{m} \pm 53\mu\text{m}$ ,  $p < 0.01$ ) and the ITD of DZOS twins ( $853\mu\text{m} \pm 202\mu\text{m}$ ,  $p < 0.05$ ).

**Conclusion:** The reproducibility of palatal intraoral scans proved to be excellent. The morphology of the palate shows differences between members of MZ twins despite their almost identical DNA, indicating that this method could be useful in forensic odontology.

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### Palatal Rugae, Human Identification, Intraoral Scanner

## G14 Postmortem Anterior Tooth Loss and Time Since Death

Susan J. Baker, DMD\*, Atlanta Laser Periodontics & Dental Implants, Norcross, GA 30092; Murray K. Marks, PhD, University of Tennessee, Knoxville, TN 37920; James M. Lewis, DMD, Madison, AL 35758

**Learning Overview:** After attending this presentation, attendees will understand the merits and limitations of estimating time since death by human anterior tooth loss in found remains. Knowledge will be gained regarding periodontal ligament during decomposition phases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aiding death investigators and forensic odontologists in determining the significance of missing or retained anterior dentition in found remains and by furthering knowledge of periodontal ligament decomposition.

**Materials and Methods:** This study examined human periodontium decomposition in 20 dentitions from donated bodies at the National Forensic Academy's rural Cumberland Forest Decomposition Facility near Oak Ridge, TN. Jaws were photographed, radiographed, gingival margin and periodontal probing depths recorded, attachment level calculated, mobility charted, occlusal wear scored, and thin sections prepared for light microscopy.<sup>1,2</sup> Daily weather conditions and seasonality were recorded.

**Results:** As expected, placement during warm and wet/humid conditions with average temperatures between 70°F and 95°F and daily rainfall averaging three millimeters (mm) or more daily resulted in greater insect (i.e., blowfly [Calliphoridae]) activity and more rapid gross soft tissue decomposition. Placement during warm and dry conditions with daily rainfall averaging less than 3mm resulted in gross mummification (desiccation) and affixed, stationary soft tissues. Cool and wet conditions with average temperatures between 40°F and 70°F had delayed soft tissue decomposition and postmortem loss. Placement during cool and dry conditions with average daily rainfall less than 3mm delayed decomposition resulting in desiccation. Postmortem tooth loss chronology corresponds with periodontal health during life. Advanced periodontal disease, increased mobility, and alveolar recession recorded at body placement showed accelerated postmortem tooth loss. The loss of the surrounding keratinized gingival tissues and the alveolar crest fibers of the Periodontal Ligament (PDL) contributed to periodontal decomposition and tooth loss. Insect larval activity did not influence subcrestal PDL decomposition as PDL width varies from 0.1mm–0.4mm and first instar blowfly larvae are about 2mm in length and 0.75mm in width.<sup>3</sup> An expected delay in tooth loss in individuals with occlusal wear in a healthy dentition was not witnessed. Since a comparative histological study of the healthy and decomposition PDL is lacking, the assumption of past researchers that the periodontal ligament space is “empty” is erroneous. This study demonstrates a long-lasting remnant of the collagenous fibers during various stages of decomposition. In nine subjects, a decrease in mobility of one or more teeth was noted over time. Desiccation of the PDL was thought to aid in mobility decrease and tooth retention.

**Conclusion:** Length of time since death does not appear to be able to be determined due to anterior tooth loss. Considering area temperatures and humidity levels may aid in narrowing the time frame.

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### Time Since Death, Postmortem Tooth Loss, Periodontal Ligament

## G15 Several Key Concepts in Bitemark Analysis Are as Relevant Now as Ever

*Richard R. Souviron, DDS\*, Coral Gables, FL 33134; Leslie A. Haller, DMD\*, Miami, FL 33131*

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**Learning Overview:** The goals of this presentation are to discuss the difference between evidence and investigative information with respect to bitemark analysis and to discuss several key concepts of bitemark evidence that can contribute valuable evidence and information to legal investigations.

**Impact on the Forensic Science Community:** There has been a great deal of controversy surrounding bitemark evidence. Some want to exclude any and all bitemark analysis from being used as evidence. This presentation will impact the forensic science community by offering aspects of bitemark analysis that are of value to the legal system as evidence and investigative information, despite the misuse and mistakes of the past.

This presentation involves the terms evidence and investigative information and the difference between the two. Yourdictionary.com defines *Evidence* as the available body of facts ... indicating whether a belief is true or valid. Dictionary.com defines *Investigate* as to carry out a systematic or formal inquiry ... so as to establish the truth.

Several bitemark cases will be presented that illustrate the value of bitemark analysis and the difference between investigative information and evidence. The importance of this analysis of bitemarks and possible profiling may provide valuable investigative information for the exclusion of suspects or a suspect. The cases highlight incidents involving exoneration of a suspect, scene photos versus bias, elimination of suspects, evidentiary value, inability to eliminate a suspect, and corroboration of statements by those involved in a case.

Attendees will learn the difference between bitemark analysis and bitemark comparison as described in a previously published paper.<sup>1</sup> The objective is to address the evolution of bitemark analysis and evidence rules and guidelines over time highlighting what should not be done and what should be kept as valuable input to the criminal justice system both as evidence and as investigative opinion.

### Reference(s):

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### Bitemark, Evidence, Investigative Information

## G16 Dental Age Estimation: A Comparison of Three Methods of Estimating Dental Age in a Population of Kuwaiti Children, Adolescents, and Young Adults

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**Learning Overview:** The goal of this presentation is to evaluate the mean age difference between Chronological Age and Dental Age (CA-DA) using the Simple Average Method (SAM), the London Atlas method, and Moorrees data of tooth development stages, in addition to measuring agreement between the proposed three methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence of the DA assessment method accuracy in comparison to CA. This may influence the decision of which dental age assessment is to be used in certain situations. Decision-making is based on the accuracy of the method, its applicability, and how quickly it can be applied.

**Background:** There has been an increase in the demand for age assessment in the living due to illegal migration.<sup>1</sup> Dental Age Estimation (DAE) is a surrogate for CA as it is the most accurate of the biological markers used to estimate the age of individuals.<sup>2</sup>

The DA of Kuwaiti children and adolescents was estimated using the SAM utilizing all teeth present in the maxilla and mandible on the left side, the Atlas Method to assign an “age” to a subject utilizing the Atlas schemes, and the Moorrees method assessing the ten teeth described in the original paper and with the numerical data derived by “reverse engineering.”<sup>3-5</sup> The DA estimated by these three methods was compared to the CA.

**Patients, Materials, and Methods:** The sample was Dental Panoramic Tomograms (DPTs) from the archives of Kuwait Ministry of Health Dental Hospitals comprising 402 healthy children aged between 5 and 15 years. Exclusion criteria were unsatisfactory images, presence of dental anomalies such as tooth agenesis or supernumerary teeth, and a medical condition that may be deemed to influence dental development. In addition, non-Kuwaiti nationality subjects were excluded from the study.

Each of the anonymized DPT was assessed in random order and personal details masked from the observer.

SAM and the Moorrees methods assessed dental age utilizing Draft Dental Age Quicksheets.<sup>6</sup> The London Atlas method matches DPT radiographs as closely as possible to one of the 23 schematic drawings.

**Ethical Approval:** This was granted by The Standing Committee for Co-ordination of Medical Research at the Ministry of Health, the State of Kuwait—Certificate #899/2018.

**Reference Data Set 2:** This consisted of 1,393 Kuwaiti children, adolescents, and young adults comprising 801 females aged from 2.80 years to 25.8 years and 586 males aged 3.23 years to 25.77 years.

**Results:** Intra- and inter-examiner agreement were calculated using Cohen's Kappa with scores of 0.76 or above which is “good to excellent” agreement.

The CA minus DA difference is an overestimate of 0.39 years for females and an underestimate of 0.14 years for males. The London Atlas overestimated the CA by 0.60 years for females and 0.19 years for males. Moorrees technique gave an underestimate of 1.01 years for females and 0.89 years for males. Bland-Altman plots showed an even distribution of difference around the CA.<sup>7</sup>

**Conclusions:** The SAM technique is the most accurate with a two-to-three-month CA Minus DA difference. The Atlas method was less accurate. The Draft Quick Sheets makes the implementation of SAM a rapid procedure. This makes the SAM the favored technique for DAE in a forensic science setting where ethnic-specific reference data are important.

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### Dental Age Estimation, Dental Age Assessment, Kuwaiti Reference Data Set

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## G17 The Impact of Age Estimation of Recruited and Exploited Children by Terrorist and Violent Extremist Groups

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**Learning Overview:** After attending this presentation, attendees will be informed about the importance and value of estimating age based on a methodological approach in the context of terrorist attacks involving recruitment of children and adolescents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the legal implications of age estimation of recruited and exploited children by terrorist and violent extremist groups.

Age estimation is the application of dental, skeletal, and physical maturity indicators in assessing the most probable chronological age of individuals.

Terrorist attacks, armed conflict, and all other forms of violent extremist groups are influenced by the age group of the recruited children–adolescents (7–21 years). A child can be a victim of an armed conflict or an alleged offender; however, the age assessment approach should be used efficiently to estimate the age, to classify the age range, then to promote the protection, rehabilitation, and reintegration of the child in society. The arrest of a child perpetrator takes precedence over the identification of a child perpetrator who has died while carrying out terrorist activity.

Situations in which a child who has not reached the minimum age of criminal responsibility is held liable or where a minor is prosecuted at the same standard of criminal responsibility as an adult must be avoided. Therefore, age estimation should be conducted as closely as possible to the time of the incident, as opposed to the time of the arrest.

The legal system has increased its efforts to combat terrorism and extremist groups. Adhering to the minimum age of criminal responsibility is crucial when it comes to underage recruitment and child trafficking, due to the inherent margin of error of age estimation. Globally, there is an irrefutable legal presumption that a child under a certain age is incapable of committing a criminal offense.

The forensic scientist should be aware of the country-specific legal significance of certain age groups such as 7, 15, 18, and 21 years in the context of criminal liability, the legal working age, and child trafficking. The use of unverified examination methods for age estimation can have dire consequences in court. The juvenile legal system stipulates the enrollment in a correction facility as a means of guiding, educating, and rehabilitating the minor. Alternatively, the child remains in the care of his/her parent(s) or legal guardian(s), provided that the aforementioned sign an affidavit, asserting responsibility for the prevention of the child's involvement in future terrorist activity.

The dignity of the child always takes precedence; therefore, the least invasive method of age assessment must be employed. Independent professionals carrying out the age assessment must comply with international human rights standards. More recently, new developments are focusing on digital apps that can be used in age assessment (e.g., SALEM mobile app).

The emphasis falls not on revealing the identity of the child, but rather on leading the child back toward society by virtue of the correct application of internationally approved guidelines.

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### Age Estimation, Juvenile/Adult Legal System, Human Rights

## G18 The Applicability of Caucasian and Chinese Dental Reference Datasets for Age Estimation in Hispanic Children in Texas

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of accuracy of the estimated age using two ethnically different dental reference datasets in Hispanic children. This will be presented in the form of a research study aimed at assessing the age of children of Hispanic origin in south Texas using the United Kingdom Caucasian and Hong Kong Chinese dental reference datasets.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of ethnic variations in dental age estimation. Attendees will learn how ethnic variations could influence the accuracy of estimated age by conducting dental age estimation using two ethnically different reference datasets in children belonging to Hispanic ethnicity in south Texas.

Dental development has been used as a forensic dentistry tool to estimate the age of subjects involved in falsified age claims and those without authentic birth documentation. The United States Border Agency reported a recent increase in the number of Unaccompanied Alien Children (UAC) intercepted in the United States' southwest border. The majority of children belong to Hispanic ethnicity originating from Central and South American countries. To date, no dental age estimation method has been evaluated for applicability in a broader age range of children of Hispanic origin.

This study aimed to assess the applicability of United Kingdom Caucasian and southern Chinese dental reference datasets for age estimation in Hispanic children in Texas. Dental panoramic radiographs of 120 healthy children constituting 60 females and 60 males aged 6 to 17 years were obtained from the University of Texas Health San Antonio School of Dentistry archives. All the teeth in left side maxillary and mandibular arches were scored according to Demirjian's classification of tooth development stages (A to H). Chronological Age (CA) was calculated from birth date and the date of exposure of radiograph. To calculate the Dental Age (DA), the mean age (x) corresponding to each tooth's development stage was obtained and averaged from the Caucasian and Chinese dental reference datasets, separately for females and males. Statistical significance was set at  $p < 0.05$ , and paired sample *t*-test was used to compare the difference (CA-DA) between CA and DA obtained from the Caucasian and Chinese reference datasets. The Caucasian reference dataset overestimated the age of Hispanic females and males by 0.60 years and 0.42 years, respectively. Similarly, the Chinese reference dataset overestimated Hispanic females' and males' age by 0.84 years and 0.49 years, respectively. The difference was not statistically significant, except for Hispanic males using the southern Chinese dataset ( $p < 0.05$ ). In females, the estimated age (CA-DA) difference ranged from -1.82 to +0.68 years using the Caucasian dataset and -3.02 to +0.36 years using the Chinese dataset. In males, the difference ranged from -1.66 to +1.40 years for the Caucasian dataset and -2.71 to +1.74 years for the southern Chinese dataset. It is concluded that, on average, both Caucasian and Chinese dental reference datasets overestimated the age of Hispanic males and females by over six months. These inaccuracies indicate the very strong need for a Hispanic-specific dental reference dataset.

### Age Estimation, Hispanic, Children

## **G19 Third Molar Development Stages on Panoramic Radiographs Differ From Those on Cone Beam Computed Tomography (CBCT)**

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**WITHDRAWN**

## G20 First Molar-to-Tooth-Length Ratios in Age Estimation

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**Learning Overview:** After attending this presentation, attendees will better understand a simple and easy-to-use new method for estimating age in developing dentitions and its advantages over a standard dental age estimation method. This will be presented in the form of a research study that aimed to compare the new technique to an existing standard method of dental age assessment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the steps of the new, alternative procedure for age estimation and presenting its advantages over an existing standard dental age estimation method in terms of age correlation, error rate, and recording tooth development.

Permanent teeth development is crucial to assess age in living children, adolescents, and individuals who have just crossed the threshold of legal adulthood. The literature is replete with a variety of methods of age estimation in the above age group, and this study presents yet another new technique for age estimation in developing permanent teeth; the method was designed to compare its correlation to age and accuracy of age estimation (in terms of error rate) with a standard dental age estimation method.

Since the first molar is the first permanent tooth to commence development and complete calcification, the ratio of its length to that of the lengths of remaining teeth should consistently change with age (i.e., ratio of non-first molar tooth length to first molar length should—by and large—increase with age). The length of all eight teeth on the mandible's left side were measured on orthopantomograms of 311 subjects (219 females and 92 males) ages 9 to 23 years using a free-to-download open source image editor (GIMP version 2.10). The length of the first molar was used to divide the length of the remaining seven teeth separately to obtain seven ratios; these in turn were subjected to multiple regression analysis with them as independent variables and chronological age as dependent variable. In addition, Demirjian's grading system and its modifications were used to assess the same teeth on the same radiographs.<sup>1-3</sup> Stage- and sex-specific maturity scores assigned to the teeth were totaled and regression analysis undertaken with the total maturity score as the independent variable and chronologic age as dependent variable.<sup>3</sup> Multiple regression analysis for the seven ratios produced  $R = 0.81$  for males (Standard Error of Estimate (SEE)  $\pm 2.1$  years) and  $R = 0.78$  for females (SEE  $\pm 2.1$  years), whereas the linear regression analysis for Demirjian's total maturity scores resulted in  $r = 0.76$  for males (SEE  $\pm 2.3$  years) and  $R = 0.77$  for females (SEE  $\pm 2.1$  years).

The "ratios method" applied herein has a slightly higher correlation to age and is marginally more accurate than Demirjian's method in terms of the error rate. Moreover, it has additional advantages in that rotated teeth do not influence the measurement (tooth rotation may affect assessment of teeth using Demirjian's method), and it is not influenced by ambiguity of whether root development is completed or not, or any other subjective stage is reached or not, which Demirjian's method may be. In conclusion, the relative simplicity of, and the aforementioned advantages to, measuring tooth lengths and calculating ratios—coupled with their stronger age-correlation and lower Standard Error of Estimate—indicates that the new technique may be used as an alternative to Demirjian's method.

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### First Molar Length Ratio, Demirjian's Method, Regression Analysis



## **G21 An Evaluation of Cameriere’s Tooth Development Measurements on Panoramic Radiographs and the Effect on Related Age Prediction**

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**WITHDRAWN**

## G22 Demirjian's Dental Age Estimation Using Third Molars: A Systematic Review

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**Learning Overview:** This presentation aims at educating attendees as to the way of conducting literature searches and qualitative analysis in a systematic review. Attendees will learn whether third molar maturation, which is routinely studied for the purpose of age estimation, is a valid and reliable indicator of age.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by presenting substantial proof related to the accuracy and reliability of third molars as indicators of whether an individual has attained the legal age of majority and will thus aid forensic anthropologists and medicolegal professionals across the globe in deciding whether to use Demirjian's scoring method for investigating third molar maturation in routine use.

Forensic age estimation in the living has paramount medicolegal and social implications in the modern world. It is a crucial aspect of identification in both civil and criminal law and is routinely conducted in individuals seeking asylum, unaccompanied minors, and refugees without proper documentation indicative of their identity. It is conducted in individuals participating in sports events, applying for jobs, pensions, etc. It is conducted before assigning criminal responsibility and in victims of child solicitation, pornography, etc.

Most of the methods of age estimation are based on observing appearance, ossification, or fusion as an indicator of skeletal maturity. These indicators being used for age estimation usually attain complete maturity by the age of 18 years and, hence, are ill-suited to define adulthood and legal maturity. Third molars usually erupt between the late teens and early 20s and thus can be used as markers to discriminate the age of an individual as being 18 years. Multiple studies investigating the use of third molar development for age estimation have been conducted, and most of these studies have used Demirjian's eight-stage scoring method. This method divides third molar maturation into eight distinct stages that can be observed in an orthopantomogram. However, as third molar eruption is quite inconsistent and unpredictable, a systematic review was planned by this study that aimed at verifying the accuracy and reliability of third molars as indicators of the age of an individual when their development has been graded using Demirjian's scoring system.

The present systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under CRD42018099603 and included a methodical search across four databases for articles that answered the review question, "Are third molars accurate and reliable indicators of the dental age estimated using Demirjian's method?" This exhaustive search resulted in acquiring 1,402 articles that were screened down to ten articles after going through the titles, abstracts, and full texts of these 1,402 articles. The ten articles acquired after this arduous searching process were then subjected to qualitative synthesis using the QUADAS 2 tool, where they were observed to be devoid of a high risk of bias. The results of these studies were compared to seek the answer to the review question. It was observed that the third molar maturation, when scored using Demirjian's rating system, were statistically significantly correlated with the chronological age of participants across all the studies. Furthermore, it was observed that Stage H of Demirjian's scoring system showed a likelihood of an individual being more than 18 years of age that ranged from 75% to 100%. It was also observed that across all the studies, the difference between estimated and chronological ages of all the individuals ranged from 0.01 years to 1.5 years. The systematic review thus concluded that third molar maturity when analyzed using Demirjian's eight-stage scoring system is an accurate and reliable indicator of dental age.

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### Age Estimation, Third Molar, Demirjian's Method

## H1 Pediatric Sudden Unexpected Death (SUD) Due to Undiagnosed Mediastinal T-Cell Lymphoblastic Lymphoma: A Series of Three Cases

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**Learning Overview:** After attending this presentation, attendees will understand the pathophysiology and presentation of pediatric mediastinal T-cell lymphoblastic lymphoma and its diagnosis at autopsy.

**Impact on the Forensic Science Community:** Per this study's research, this is the first case series of undiagnosed mediastinal T-cell lymphoblastic lymphoma leading to SUD in children. This presentation will impact the forensic science community by showing that while pediatric SUD secondary to malignant mediastinal masses is rare, timely diagnosis and treatment may prevent life-threatening complications or death. Mediastinal T-cell lymphoblastic lymphoma, in particular, may be rapidly progressive and potentially fatal if not detected early.

SUD in childhood is a rare phenomenon with a prevalence of approximately 1.4 deaths per 100,000 children. Undiagnosed neoplasms in previously healthy children are rare causes of SUD, with most cases involving the heart or central nervous system.<sup>1</sup> The literature on pediatric SUD due to unrecognized mediastinal neoplasms is limited to a small number of case reports with several cases of mediastinal neoplasms confirmed to be secondary to T-cell lymphoblastic lymphoma.

Lymphoblastic Lymphoma (LBL) is the second most common type of non-Hodgkin lymphoma in childhood. The majority of LBL cases are of T-cell origin. Although T- and B-cell LBL are morphologically indistinguishable, the clinical presentation can potentially help differentiate the two entities. A mediastinal mass is the most typical manifestation of T-cell LBL. Mediastinal involvement is often massive, with a bulky enlargement associated with bilateral pleuro-pericardial effusions.<sup>2</sup> The development of the mass in a limited space results in progressive airway obstruction and can lead to Superior Vena Cava (SVC) syndrome. The clinical presentation is non-specific, with a predominance of respiratory symptoms that are virtually indistinguishable from common respiratory conditions.<sup>3</sup> However, the symptoms are more apparent when the patient is supine and may improve in either the sitting or prone positions.<sup>4</sup> This study describes three cases of previously healthy pediatric patients who suffered SUD attributed to autopsy-diagnosed anterior mediastinal T-cell lymphoblastic lymphoma.

In Case 1, a 2-year-old girl presented with nine days of cough and dyspnea. She was diagnosed with croup, but her symptoms progressively worsened, and she was transferred to a tertiary care center with the diagnosis of status asthmaticus. On admission, she was found in cardiorespiratory arrest. Postmortem examination revealed a firm rubbery mass surrounding the heart, great vessels, and lungs, compressing the bronchi. In Case 2, a 3-year-old girl had been suffering from a respiratory tract infection over several days. On the day of her death, she complained of neck pain and dyspnea. Soon after, the patient went into cardiorespiratory arrest. Autopsy revealed a firm nodular mass in the anterior mediastinum compressing the brachiocephalic trunk, left carotid artery, left subclavian artery, and the superior vena cava. Case 3 involved a 2-year-old boy who was found unresponsive on his stomach in his crib. Per his parents, the child had cold-like symptoms for several days before his death. Postmortem examination revealed a firm, rubbery anterior mediastinal neoplasm surrounding the superior vena cava, both subclavian veins, the aortic arch, and the proximal great arteries. In all three cases, the pathology of the masses was consistent with T-cell lymphoblastic lymphoma.

These three cases show the importance of identifying children with mediastinal masses that could potentially lead to life-threatening presentations and pediatric SUD. When evaluating a child with respiratory symptoms that are exacerbated in the supine position, it is important to consider an anterior mediastinal mass in the differential diagnosis. Additionally, these cases highlight the importance of considering a hematologic neoplasm at the time of autopsy in a previously healthy child who dies suddenly.

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### Pediatric Sudden Unexpected Death, Mediastinal Neoplasm, T-Cell Lymphoblastic Lymphoma



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## H2 Variations in Postmortem Perianal Findings in Infants: A Retrospective Review of Non-Traumatic Abnormalities and the Potential Diagnostic Pitfalls in Determining Trauma

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**Learning Overview:** The goals of this presentation are to provide a reference for the forensic examiner to aid in an accurate and systematic approach to identifying non-traumatic perianal abnormalities of infants and to demonstrate the potential diagnostic pitfalls in determining the presence of trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the ability to distinguish between non-traumatic and traumatic perianal findings in postmortem examinations of infants.

The forensic pathologist's objectives in performing postmortem examinations include determining the cause and manner of death and identifying and documenting injuries, evidence of abuse, and neglect; these are particularly crucial in the deaths of vulnerable individuals, such as the elderly and young children. Postmortem examinations of children, particularly infants, require an additional level of caution when interpreting findings due to the susceptibility of this demographic to abuse and the medicolegal implications involved. Examination of the anogenital region is of particular importance in that identification of injuries in this area may suggest the possibility of sexual abuse and prompt additional intervention, especially when other children remain in the household.

Identification of anogenital injuries and evidence of abuse in antemortem and postmortem settings may be complicated by a variety of non-abuse related conditions, lesions, or congenital abnormalities. For example, a history of recent constipation or diarrhea, diaper rash, infection, and Hirschsprung's disease can all present with findings that mimic signs of abuse. The postmortem examination of the anogenital region is further complicated by postmortem changes that mimic traumatic or exaggerate non-traumatic abnormalities, raising suspicion for trauma. These changes include dilatation of the anus, augmentation of perianal fissures secondary to anal dilatation, and discoloration of the anogenital skin and mucosa. Anal dilatation, in particular, is a common non-specific postmortem finding, especially in infants. It may be the result of postmortem relaxation of the anal sphincter or it can represent previous anal penetration; therefore, proper identification and interpretation of such findings is necessary.

Photographic documentation is an essential component of the postmortem examination and serves as a digital collection of evidence. In this study's office, examination of unexpected or unexplained infant deaths includes photographic documentation of the anogenital region. In order to document the appearance and prevalence of non-traumatic perianal abnormalities, and to correlate their incidence with additional investigative and autopsy findings, a database of infant autopsy photographs was created. This database includes the perianal photographs of both male and female infants one month to six months of age, taken over a ten-year period from 2010 to 2020. Images will be presented that best highlight the variations in non-traumatic postmortem perianal findings. Photographic examples of findings due to sexual trauma will also be presented for comparison.

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### Postmortem Findings, Child Abuse, Autopsy

## H3 Recognizing the Misuse of Probabilistic Language (PL) and False Certainty in False Accusations of Child Abuse

Steven C. Gabaeff, MD\*, Healdsburg, CA 95448-7058

**Learning Overview:** The goal of this presentation is to increase awareness of the misuse of PL, false certainty statements, and their impact of false allegations of child abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and evidentiary rulings regarding false certainty statements in court.

PL is language used to convey mathematical probabilities in narrative form. Terms like “concerning for,” “highly likely,” “never,” and “suspicious of” are common PL. PL play a large and previously unanalyzed role in child abuse prosecution and particularly false accusations of child abuse; a common occurrence. PL can be used in conformance with principles elucidated in forensic epidemiology or systemically misused with intentional imprecision, when not justified, to promote a misdiagnosis of abuse, with dire consequences.<sup>1</sup> The application of actual probability analysis using tested mathematical models, like Bayes Theorem, is essential to an analysis of the actual probability of abuse, in a specific case, to avoid false accusations of abuse.

Bayes Theorem, first published in 1764, is a calculation of actual odds (“posterior odds”) of an event (like abuse) or a result (like having a disease), being a true positive, based on mathematical probabilities that are known.<sup>2</sup> It is imperative to remember that the result being calculated is a mathematical probability, not a certainty. The posterior odds are computed with the consideration first, of the “prior odds” of an event (abuse) occurring in a defined population of subjects (i.e., What is the rate of abuse among professional caregivers?). Defined populations can be general (all caregivers) or specific (i.e., military families, professional caregivers, foster parents, etc.). The prior odds are then multiplied by calculations of the reliability of an “indicator” or criteria (i.e., a lab result, an imaging finding, or a physical finding, like a bruise or petechiae). The magnificence of Bayes Theorem is the simplicity in which an infinite variety of subpopulations, with different prior odds, can be conjured up and used with a wide variety of indicators, each with its unique reliability. Reliability is calculated using variants of conventional sensitivity and specificity which, when compared as a ratio, create a “likelihood ratio.” The likelihood ratio is a reflection of the reliability of an indicator to deliver the correct decision. Indicators can be very reliable evidence, like DNA matches, that generally help to prove the event (abuse) occurred. Indicators, on the other hand, when analyzed, can comport with forensic standards defined as non-specific and unreliable. Using mathematics, Bayes brings clarity to subjectivity and/or potential bias, reflected in imprecise “false certainty” statements. In reality, when studied, the indicators used in modern child abuse to diagnose disputed abuse cases are uniformly quite unreliable. This calculated unreliability results in very low posterior odds that generate calculated probability and reliability that abuse occurred under 1%.

With evidentiary standards beginning at 50% reliable in certain court proceedings, and rising in criminal matters to >95%, to have evidentiary value, low calculated probabilities using Bayes suggest that false certainty statements, about unlikely hypothesis, create opportunities for injustice. In contested abuse cases without witness evidence, imprecise probabilistic language is widely used as evidence. With the pediatric abuse literature and fund of knowledge of child abuse pediatrics now widely questioned by independent scientific analysis, all forms of imprecision take on the character of false certainty statements. After probability analysis using Bayes, it is manifestly obvious that such imprecision and false certainty have become the *sine qua non* of accusatory expert opinion. The suppositious statements of false certainty that are used, currently, only detected by scientists and physicians with the requisite advanced knowledge of these issues, expose deep flaws in the practice of child abuse pediatrics. On balance, there is an emerging reality that the collective suffering of falsely accused families dwarfs the horrific impacts associated with real abuse and exposes that iatrogenic abuse may be the most common form of child abuse in the legal system. Furthermore, a false accusation of child abuse is child abuse.

The misuse of PL to convey false certainty and its ramifications for innocent caregivers must be acknowledged as a first step in eliminating false accusations of child abuse.

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### Child Abuse, False Accusations, Probabilistic Language

## H4 Fatal Systemic (Paradoxical) Air Embolism Diagnosed by Postmortem Funduscopy

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**Learning Overview:** After attending this presentation, attendees will be able to identify distinctive retinal air emboli associated with systemic air embolism.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing the necessity for dedicated postmortem fundal examinations.

Due to its uncommon occurrence, few clinicians or pathologists have seen patients or decedents with systemic air embolism. Likewise, its clinical signs—skin marbling (*livedo reticularis*), lingual pallor (Liebermeister’s sign), frothy arterial bleeding, and Retinal Air Emboli (RAE)—are not well known. The millwheel murmur characteristic of venous air embolism is typically absent in arterial air embolism. The unique fundoscopic findings of RAE include conspicuous intravascular air columns and bubbles and pale silvery streaks representing spectral glare highlighting air-filled retinal blood vessels.

**Case Report:** An 8-month-old infant with a distended abdomen arrived at the emergency department *in extremis*. Found unresponsive at home, resuscitative efforts were unsuccessful, with death pronouncement occurring 65 minutes after the public safety answering point call. Five hours after death, postmortem indirect and direct ophthalmoscopy detected a few scattered splinter retinal hemorrhages over the posterior poles and distinctive RAE—intravascular air emboli interspersed between blood columns and silvery outlines of retinal blood vessels. Postmortem radiography disclosed intravascular air, subcutaneous emphysema, rib fractures, and a pneumoperitoneum. Autopsy confirmed those findings and detected cerebral and cardiac intravascular air bubbles as well as severe abdominal trauma with hepatic, pancreatic, mesenteric, and duodenal/jejunal lacerations with resultant pneumo-hemoperitoneum. A 1.5cm transmural tear in the inferior vena cava’s ventral wall allowed air into the right heart and a patent foramen ovale permitted systemic (paradoxical) air embolism.

Categorized as venous, arterial, or paradoxical, air embolism is infrequent but can be catastrophic with significant morbidity and mortality. It has occurred following penetrating or blunt trauma, pulmonary barotrauma, surgical procedures, vascular catheterization, intraosseous infusion, hemodialysis, mechanical ventilation, bronchoscopy, pulmonary fine-needle aspiration, endoscopy, placenta previa, Caesarean section, criminal abortion, and intra-operative blood recovery. Descriptions of RAE in published case reports and case series are scarce and none contains photographic images of RAE.<sup>1-4</sup> The first recorded case with depictions of RAE was in 1914 by Wever who illustrated air bubbles and “silvery glittering rods” within the retinal vasculature of a 25-year-old man with pulmonary abscesses who died suddenly following a pulmonary vein erosion during Paquelin thermocautery.<sup>5</sup> Subsequent line drawings or artistic renditions of RAE have occurred in four case reports—a non-fatal case following maxillary sinus irrigation published in 1920, two occurrences associated with suboccipital pneumoencephalography described in 1957 and 1958, and a fatality during laser bronchoscopic resection of a bronchogenic carcinoma reported in 1988.<sup>6-9</sup>

While there are a few descriptions and illustrations of RAE, there are no published photographic images of RAE in infants, children, or adults. This case report describes and photographically documents classic RAE associated with fatal systemic (paradoxical) air embolism. These crucial retinal findings emphasize the importance of consistent and thorough postmortem fundal examinations.

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### Retinal Air Emboli, Air Embolism, Funduscopy

## H5 The Aftermath of Abusive Head Trauma: A Unique Pediatric Disease

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**Learning Overview:** The goals of this presentation are to: (1) list the anatomic manifestations of abusive head trauma; (2) describe the long-term clinical and pathological complications of abusive head trauma; and (3) discuss the significance of long-term consequences with respect to pathophysiology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting this study that elucidates a disease complex unique to all other diseases of children.

**Background:** Abusive head trauma is associated with a high mortality rate. In those who survive, neurological complications are numerous and severe. In one study of 837 cases, 59% of the survivors of abusive head trauma had moderate to severe disability and 5% were in a persistent vegetative state.<sup>1</sup> The pathophysiology of the extensive brain damage in abusive head trauma is unclear.

**Methods:** A total of 13 long-term survivors of clinically suspected or confirmed abusive head trauma were identified who presented for forensic autopsy between 2017 and 2020. Cases were selected for examination on the basis of the medical information provided. Cases were not selected based on the anatomical findings or the assessment of cause and manner of death. One case did not have fixed brain available for detailed neuropathological examination and was excluded. One case of suspected post-traumatic epilepsy was due to abuse as an adult and was excluded. Brain (all cases) and dura mater (all but one case) were fixed in formalin for approximately two weeks. Post-fixed tissue was photographed, sectioned, and sampled for histopathology. Samples were dehydrated in graded ethanol and xylene solutions, embedded in paraffin, and stained with hematoxylin and eosin.

**Results:** The decedents ranged in age from 5 years to 44 years. Survival time ranged from 4 to 43 years. All decedents were severely neurologically impaired during life, requiring 24-hour care. Nine decedents were said to have “cerebral palsy.” Dura mater showed subdural fibrosis and/or a thin neomembrane in 11 of 13 cases. Focal areas of osseous metaplasia of the dura were present in three cases. Brain examination showed widespread ischemic brain injury in 12 of 13 decedents, typically involving vascular territories of the anterior circulation. The territory of the posterior cerebral artery was inconsistently involved. The changes were patchy in all cases when present and asymmetric in five cases. There were more severe changes over the superior convexities and parasagittally, although generally in a vascular territory distribution rather than an arterial border zone distribution. There was frequent sclerosis of the basal ganglia, thalamus, and cerebellum, and hippocampal sclerosis in a minority of cases. One decedent had callosal agenesis and migration disturbance, suggesting that the severe impairment was developmental rather than secondary to abusive head trauma.

**Conclusions:** Abusive head trauma sets up a pathophysiological cascade that appears to be unique among pediatric diseases. Although the common manifestations of subdural hematoma and retinal hemorrhage are well known, extensive damage to the substance of the brain is less understood and appears to be responsible for long-term morbidity and mortality. Such damage is not explainable on the basis of an expanding intracranial hemorrhage or global ischemic (“hypoxic-ischemic”) brain injury from systemic cardiovascular collapse. The pattern of brain injury after abusive head trauma instead suggests severe vascular territory ischemia induced by blunt force trauma, which, in the absence of large vessel thrombosis, suggests that vasospasm may play a critical role in the morbidity and mortality in this setting.

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### Abusive Head Trauma, Shaken Baby Syndrome, Chronic Ischemic Brain Injury



## H6 Child and Adolescent Suicide: A Ten-Year (2010–2019) Retrospective Analysis of Medical Examiner Cases in Kentucky

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**Learning Overview:** After attending this presentation, attendees will understand the trends of suicide-related deaths during childhood and adolescence during a ten-year period and how they correlate with those found at the national level.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by identifying trends in suicide methodology, in addition to at-risk populations, in order to benefit future analytical and preventive efforts. The prevention of childhood suicide and the need for further therapy and follow-up are also discussed, as evidenced by the data reviewed.

**Introduction:** In 2018, more than 48,000 suicides were reported, becoming the tenth-leading cause of death in the United States. A Centers for Disease Control and Prevention (CDC) Vital Signs report found that, between 1999 and 2016, rates of suicide increased by more than 30% in half of the country. More than 6,800 suicides were registered in 2018 and was the second leading cause of death in adolescents (596) and young adults (621). Suicides comprise approximately 13% of the caseload at all regional offices under the Kentucky Office of the State Medical Examiner. A better understanding of the methodologies utilized and the demographics of those involved, in addition to a comparison with previously published data, can provide valuable information and guide recommendations concerning suicide prevention.

**Materials and Methods:** A ten-year retrospective review of suicide cases of children and young adolescents aged less than 18 years was conducted at the four regional offices of the Kentucky Office of the State Medical Examiner. The study focused on cases of suicide investigated by that office from January 1, 2010, through December 31, 2019. The age, race, and sex of each victim, as well as cause of death and time of year, were recorded. The investigation included analysis of postmortem toxicological findings.

**Results:** From a total of 24,662 medical examiner cases from all 120 Kentucky counties, 3,214 cases were classified as suicide after a thorough postmortem examination, with subsequent comparison to a previous review conducted by the Kentucky Office of the State Medical Examiner office from 1993–2002. The 131 (13.04%) suicides among ages 10 to 18 years were further subdivided into 12 decedents from ages 10 to 13, 96 decedents from ages 13 to 17, and 23 decedents aged 18.

The majority of decedents in all groups were males (72.7%). By racial composition, 80.68% were Caucasian, 11.90% were Black, and 8% were from other races. Very few Black females committed suicide, consisting of only 4% of victims in all groups. The number of suicides was highest in March (19.76% of cohort) and lowest in August (2.32% of cohort).

Firearm injury was by far the most common method of suicide for child and adolescent age groups, accounting for 70 cases (53.43%). Hanging (37.40%) and substance abuse (3.81%) were also leading methods of suicide.

Compared to the previous review (1993–2002), the percentage of suicides averaged across ten years increased from 12% to 13%, with the highest numbers in the 13- to 17-year age range. This study, in most respects, mirrors previous studies of adolescent suicide regarding race and sex. Regarding the method of suicide, although firearm injuries ranked first, the percentage of these compared to the last study dropped from 72.2% to 53.43%. Hangings remained the second cause for both groups, but the percentage increased from 22.2% to 37.4%.

**Conclusions:** In summary, the sex, race, and method of suicide were interestingly similar between the adolescents ages 10 to 18 years in this study of medical examiner-investigated cases of suicide. In the current study, firearm injury was the primary cause of death in most decedents in all groups, which was similar to the previous study (1993–2002) conducted.

Suicide is a critical public health problem in the United States that can have long-lasting adverse effects on individuals, families, and communities. An integrated coroner/medical examiner system profits from more comprehensive data retrieval of such cases and analysis for better public health awareness. Overall, forward-looking efforts based on new evidence and past accomplishments are needed to reduce the incidence of suicide and suicide attempts by influencing mitigation and prevention strategies.

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### Suicide, Child, Adolescent





## H7 When Epidemic Meets Pandemic: A Case Series of Pediatric Fentanyl Deaths in Denver, Colorado

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**Learning Overview:** The goals of this presentation are to identify the current fentanyl trends in the City and County of Denver and analyze the impact of the pandemic on fentanyl trends in the pediatric population through case examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by motivating attendees to recognize the current trends and support implementation of programs aimed at appropriate childcare and injury prevention.

The opioid epidemic continues during the COVID-19 pandemic, and fentanyl use is having an increasing impact on the health of the pediatric population of the City and County of Denver.

This study is intended to report the incidence of deaths involving fentanyl and fentanyl analogs in the City and County of Denver from January 2017 to September 2020 in the pediatric age group (aged less than 18 years) and to specifically illustrate the impact of the current fentanyl trends on pediatric deaths during the COVID-19 pandemic using three case studies.

In 2017, a public health emergency was declared by the United States Department of Health and Human Services in response to a relentless increase in opioid-related deaths in the United States.<sup>1</sup> Deaths involving synthetic opioids, primarily fentanyl and fentanyl analogs, continued to rise in 2018, and over 31,000 people died from overdoses involving synthetic opioids (other than methadone).<sup>2</sup> Data from the Denver Office of the Medical Examiner (DOME) show a similar significant increase in the number of fentanyl deaths since 2017.<sup>3</sup> In 2020, these trends have not only continued, but have grown exponentially and are beginning to show a greater impact on younger age groups.

**Methods:** All deaths reported by the DOME with fentanyl or fentanyl analogs listed as either the cause of death or contributing to death were included and analyzed according to age group and date. Toxicology was performed on antemortem or postmortem blood specimens, depending on the case.

**Results:** Of 182 cases of death involving fentanyl and/or fentanyl analogs, eight were reported in the pediatric age group of 18 years of age and younger. Seven of the eight fentanyl-related deaths occurred in 2020, including the youngest age of nine years old.

**Discussion:** This study illustrates the impact of the current opioid epidemic, highlighting the pediatric age group as an emerging at-risk demographic, particularly in the setting of the COVID-19 pandemic, and underscoring the importance of appropriate childcare and treatment programs to prevent further opioid-related deaths.

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### Fentanyl, Pediatric Death, Forensic

## H8 Infant Deaths Associated With Methamphetamine Exposure: A Case Series

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**Learning Overview:** The goal of this presentation is to compare the characteristics of infant deaths in which methamphetamine was a cause or contributing factor.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring ways an infant may be exposed to methamphetamine and the potentially complex medicolegal questions raised in these cases.

**Background:** Although the consequences of prenatal methamphetamine exposure have been explored, comparatively less is known about the effects of methamphetamine exposure in infancy.<sup>1</sup> Moreover, the toxic concentration of methamphetamine in infant blood is unknown. For infants in which methamphetamine is detected in postmortem blood, several key questions can be posed: what was the source of the methamphetamine, what was the route of exposure, and what do the postmortem blood concentrations mean? It has been reported that young children may be exposed to methamphetamine in several ways, including through food/beverage contamination, transdermal absorption, accidental ingestion, second-hand smoke, and mother's breastmilk.<sup>2-5</sup> Though there are several studies regarding methamphetamine and its role in fetal and infant deaths, limits of toxicity and lethality are unclear.<sup>6-8</sup> These complex questions are of utmost importance in the medicolegal arena. After a recent series of infant deaths in which methamphetamine was a potential contributing factor, this study aimed to identify similar prior cases.

**Materials/Methods:** This study searched a database of deaths investigated by medical examiner offices serving multiple counties in western Michigan for infant deaths in which methamphetamine may have contributed. A search was conducted for "amphetamine" in Part I (a, b, c, and d) and in Part II ("Other significant conditions") of the death certificate.

**Case Histories:** Three cases were identified in the database, all of which listed "Methamphetamine exposure" in Part II. There were no cases in which methamphetamine toxicity was an immediate or direct cause of death.

The first infant was an 8-month-old female found unresponsive in her crib with a bottle, stuffed animal, and cup. The infant was formula fed. Drug paraphernalia was found in the decedent's room at the time of the scene investigation. The father of the decedent admitted to recent use of methamphetamine. A doll reenactment showed no evidence of airway obstruction. Autopsy findings included idiopathic pulmonary hemosiderosis. Heart blood was positive for methamphetamine (11ng/mL).

The second infant was a 6-day-old female found unresponsive while bedsharing with an adult. The infant was both breastfed and formula fed. Parents admitted to methamphetamine use on the day of the infant's death. A doll reenactment was performed, and the possibility of airway obstruction could not be excluded. Heart blood was positive for methamphetamine (19ng/mL).

The third infant was a 2-month-old male found unresponsive lying supine between two adults. Reportedly, the infant was also covered by a pillow. The infant was formula fed. Marijuana plants were found in the home, but no other illicit substances or paraphernalia was found. A doll reenactment was not performed. Heart blood was positive for methamphetamine (10ng/mL).

**Results/Discussion:** In all three infant deaths, heart blood methamphetamine concentrations were at or near the reporting limit. Isomer differentiation revealed that, in all cases, 100% of the methamphetamine detected was dextro(D)-methamphetamine, consistent with the Drug Enforcement Administration (DEA) Schedule II central nervous system stimulant. In one case, the infant may have been exposed to methamphetamine via breastmilk. Notably, in two cases, an unsafe sleep environment was listed as a contributing factor, while a component of unsafe sleep could not be entirely excluded in the remaining case. Additionally, one infant had pulmonary hemosiderosis. Therefore, in all three cases, there were competing causes of death and/or contributory factors. The manner of death was indeterminate in all cases.

The question of route of exposure remains unanswered in all cases. One must be aware of pediatric developmental milestones to rule out possible scenarios and to corroborate potential reports (e.g., accidental ingestion). Such information may impact not only manner of death determination but also possible criminal charges. Additionally, it is still unknown "how much methamphetamine is too much?" The blood concentrations in these cases are among the lowest reported in deaths associated with methamphetamine; however, where the line of toxicity should be drawn remains up for debate.

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## **Methamphetamine, Infant Death, Toxicology**

## H9 The Recent Outbreak of Brorphine Use in Cook County, Illinois

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**Learning Overview:** After attending this presentation, attendees will have better knowledge regarding the epidemiology, the mechanism of action, and the toxicological features of the emergent synthetic opioid brorphine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a review of cases from a large metropolitan area where brorphine was detected in the blood specimens.

Brorphine is a novel synthetic opioid that causes psychoactive effects similar to heroin, fentanyl, and other opioids. It is structurally similar to fentanyl but different from previously identified fentanyl analogs. Synthetic opioids can be found in powder or tablet form and may be mixed with other psychoactive substances, thus increasing the risk of severe complications and even death.

A rise in the use of brorphine has been observed in Cook County IL, in the recent period. In the present study, case files from the electronic database of the Cook County Medical Examiner's Office in Chicago, IL, were reviewed from June 2020 to the present for deaths due to acute intoxication in which brorphine was identified in the postmortem samples. The following keywords were searched in the "primary cause of death" field: "combined," "drug," "intoxication," "toxicity," and "brorphine." No limits regarding age, race, and sex were imposed. Only cases in which investigative and full National Medical Services (NMS) toxicologic reports were available were included. A urine screening test for brorphine was performed in all cases. Positive cases were then confirmed by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The detected brorphine concentrations in blood specimens ranged from 0.5 to 8.1ng/mL.

The number of deaths due to synthetic opioids is rapidly increasing in the United States. Among these, brorphine use is rapidly increasing, and only little information can be found in the literature. This study adds valuable information that can help determine the current scenario of deaths due to acute toxicity in which brorphine is involved. According to the literature, as of mid-July 2020, brorphine was confirmed in blood specimens associated with fatalities in the United States and Europe. It is believed that it can cause respiratory depression alone or combined with other synthetic opioids, leading to death.

Final results, including demographic comparisons, and toxicological data, will be presented to the attendees.

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### Brorphine, Toxicology, Death

## H10 Isopropanol in Postmortem Vitreous Humor Due to Body Preparation for Tissue Procurement: A Report of Six Cases

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**Learning Overview:** The goal of this presentation is to demonstrate that isopropanol present in postmortem samples could also be attributed to body preparation for tissue harvesting prior to autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that body preparation for tissue donation is a possible cause of the presence of isopropanol in postmortem vitreous humor.

Volatile chemicals can be relevant in the determination of cause and manner of death by forensic pathologists. Isopropanol is a volatile chemical that is occasionally reported in the vitreous humor at autopsy, and its presence can be due to a variety of different causes. Isopropanol can originate from ingestion or may arise endogenously in cases of hypothermia, starvation, and diabetes mellitus.<sup>1,2</sup> There has also been a report of postmortem contamination from either embalming or tissue procurement.<sup>3</sup> This case series illustrates that isopropanol in postmortem samples can be attributed to the body decontamination process used for tissue harvesting, and this is an origin that should be considered by forensic pathologists when found at autopsy.

Six autopsy cases in which isopropanol was identified postmortem in the vitreous humor originating from decontamination of the body in preparation for tissue harvesting are presented. In these cases, organ and/or tissue harvesting was performed through Gift of Life Michigan (GOLM) between 2016 and 2020. Autopsies were performed at the Kent County Medical Examiner's Office or the McLaren Northern Michigan hospital morgue. All of the toxicology samples were analyzed at the Spectrum Health Laboratory in Grand Rapids, MI.

In these six cases, all toxicology samples were collected after tissue harvesting was completed. In four of the cases, the medical examiner collected all of the toxicology samples prior to autopsy. In one case, the medical examiner collected the vitreous humor, urine, liver, and gastric and chest cavity blood while GOLM obtained the cardiac blood prior to autopsy. In the last case, the medical examiner collected the vitreous humor and GOLM collected the femoral blood. The median vitreous isopropanol concentration in these six cases was 100mg/dL (range was 38mg/dL to 250mg/dL). In three of the cases, all other postmortem samples (i.e., blood, urine, tissue) tested were negative for volatile chemicals. One case reported an ethanol of 130mg/dl in the vitreous humor and 250mg/dL in the femoral blood, and one case had a femoral blood ethanol of 14mg/dL. The remaining case had a vitreous acetone of 6mg/dL, cardiac blood acetone of 3mg/dL, isopropanol of 3mg/dL, and a urine acetone of 18mg/dL. The liver, chest cavity blood, and gastric contents were negative for volatile chemicals. The median age was 19 years (range was 17 months to 56 years). The tissues recovered included heart valves ( $n=4$ ) and skin, bone and cartilage ( $n=2$ ), but not vitreous humor. Causes of death included gunshot wound to the head, acute fentanyl toxicity, drowning, positional asphyxia, multiple blunt force injuries, and sudden unexplained death in childhood. The manners of death included accident ( $n=4$ ), homicide, and natural.

The presence of isopropanol in postmortem toxicology can be a result of ingestion or the endogenous formation in the body. One study showed that median vitreous humor isopropanol in cases of acute isopropanol toxicity was 240mg/dL (range of 130–244mg/dL).<sup>3</sup> Given that the standard procedure for body preparation is to spray and wipe down the body with isopropanol prior to recovery, another consideration for forensic pathologists is that the presence of volatile substances is a result of the body preparation process. It is hypothesized that the surface contamination of the skin and mucous membranes by chemicals used in body preparation can lead to the passive absorption into the body resulting in the presence of volatiles in postmortem toxicology samples. A recommendation to avoid this spurious result would be for postmortem samples to be routinely collected prior to body preparation, but further research is needed to elucidate the exact mechanism of transfer.

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3. Kimberly Molina. A Characterization of Sources of Isopropanol Detected on Postmortem Toxicologic Analysis. *Journal of Forensic Sciences* 55, no 4 (July 2010): 998-1002, Doi 10.1111/j.1556-4029.2010.0136.x

### Isopropanol, Tissue Procurement, Autopsy



## H11 Paralytic Shellfish Poisoning Resulting From the Consumption of Shellfish From a Beach in Alaska

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**Learning Overview:** The goal of this presentation is to make attendees aware of an unusual form of poisoning with neurologic symptoms that can be seen through the consumption of non-commercially obtained shellfish.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that there is a need for monitoring systems to be put in place for the detection of specific neurotoxins that can arise in shellfish in various locations in coastal communities

This is a case report of a fatal case of a 62-year-old female whose death is attributed to paralytic shellfish poisoning. The woman and her family harvested blue mussels and dogwinkle snails from a beach in Dutch Harbor, AK, on July 4, 2020. After cooking the shellfish, she and her family ate them. The other members ate less than the patient. Approximately four hours later, she developed numbness, tingling in her fingers, a floating sensation, and began to vomit. Two hours later, she developed numbness from the mouth to the hands with neck and back pain. On the arrival of emergency personnel, she was suspected of having a stroke and was transported, where upon admission, she developed abnormal heart rhythm with bradycardia with subsequent cardiopulmonary arrest. The decedent's other medical history included hypertension and diabetes type 2. Given the history of exposure to personally harvested shellfish and the unusual symptoms, testing for paralytic shellfish poison was requested. Samples of the mussels, dogwinkles, and stomach contents from the postmortem examination were sent to the Alaska Department of Environmental Conservation's (ADEC) Environmental Health Laboratory (EHL). The EHL analyzed the samples using Ultra-Pressure Liquid Chromatography (UPLC) with Post Column Reaction (PCOX) and yielded the following results: dogwinkles at 287ng Saxitoxin (STX) equivalents/100g of tissue, blue mussel at 11,200ng STX-eq/100g, stomach contents at 50.2ng STX-eq/100g. For reference, the Food and Drug Administration (FDA) requires product for commercial sales to be less than 80ng STX-eq/100g. The cause of death, determined to be paralytic shellfish poisoning, was supported with the trifecta of results from the consumed product: the high level of toxicity in the stomach hours after consumption, vomiting, and the detections of five Paralytic Shellfish Toxin (PST) congeners in the urine. Unlike other states, Alaska does not perform routine monitoring of non-commercial harvesting areas, Alaska has no personal use beach monitoring programs and does not restrict personal harvesting of shellfish, which is a risky decision. Future monitoring for these potentially deadly toxins in shellfish can prevent such death and/or injury in the future.

### Paralytic Shellfish Poisoning, Saxitoxin, Neurotoxin

## H12 A Simplified DNA Barcoding Strategy for Forensically Relevant Blow, Flesh, and Scuttle Flies

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**Learning Overview:** The goal of this presentation is to demonstrate 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 building competence for DNA barcoding strategies and demonstrate performance of a simplified method designed for medicolegal death investigations. Future work will include the continued collection of local population data and elucidation of respective interspecific and intraspecific sequence variations. In addition, formal internal validations will be conducted to support casework application.

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 morphological 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 because of 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.

The strategy comprises sequencing and phylogenetic analysis of a single barcoding fragment amplified from the mitochondrial **Cytochrome C Oxidase I (COI)** locus. Using verified reference specimens, the DNA barcoding strategy is shown to enable statistically supported identification of species previously encountered in 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*. The strategy is advantageous over previous methods in the literature in that all target species may be amplified using a single primer set. Identification is demonstrated for larva and pupa collected during past HCIFS medicolegal death investigations for which species-level identification was undetermined by morphology. An ongoing project is the construction of a database of COI sequences from local specimens. This may be used to provide additional statistical analyses, in particular, the interspecific and intraspecific sequence variations, enabling comparisons to local blow, flesh, and scuttle fly populations.

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### DNA Barcoding, Postmortem Interval, Forensic Entomology

## H13 Microbiome Dynamics in *Lucilia Sericata* (Meigen, 1826) (Diptera: Calliphoridae) Developmental Stages

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**Learning Overview:** After attending this presentation, attendees will have a detailed understanding of *Lucilia sericata* microbiome diversity and dynamics throughout the developmental stages, as well as new insights on pathogens transmission by this calliphorid species.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the complexity of the results, which have both forensic and medical importance, adding novel data on necrophagous insect species' microbiome characterization.

The common green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae), constituted an experimental model in forensic and medical *in vitro* studies worldwide, as one of the first colonizers of decomposed bodies. While microbial dynamics associated to insects could bring complementary information on carcass decay evolution, this study focused on bacterial community characterization from *L. sericata* adult, immature stages and feeding substrate (swine liver), and quantitative transmission of *Salmonella enterica* (Proteobacteria) from adult to teneral specimens using Next Generation Sequencing (NGS) and quantitative Polymerase Chain Reaction (qPCR) techniques.

*L. sericata* adults were reared under constant laboratory conditions using non-inoculated liver and liver inoculated with low ( $10^2$  CFU/ml) and high ( $10^4$  CFU/ml) *S. enterica* concentrations as feeding substrate. The experiment was performed in triplicate, while insect and liver samples were collected daily.

Bacterial diversity was determined by Illumina® MiSeq® sequencing of amplified 16S ribosomal RNA (rRNA) genes of total DNA extracted from liver tissue and insect specimens. Taxonomic assignment was performed using the SILVA v138 16S rRNA database, while the community analysis and taxa Amplicon Sequence Variants (ASVs) relative abundance were performed in R.

The presence of *S. enterica* in liver tissue inoculated with low and high bacterial contents showed an increase up to  $10^{5.3}$ x as compared to the initial pathogen concentration. Meanwhile, the quantitative variation of *S. enterica* in insect specimens fed with bacterial-treated liver confirmed the pathogen transmission from adults to larval stages, to pupae, and finally to the teneral specimens. The highest bacterial abundance was reached for the third larvae stage ( $10^{5.1}$ x), with comparable amounts in the pupae stage. No *S. enterica* could be detected from the non-inoculated liver or from the insect specimens reared in the control jars.

The bacterial communities from liver tissues were dominated by Actinobacteria in the untreated samples, and by Firmicutes in the presence of *S. enterica*, with increased relative abundance in the last experimental days. The highest bacterial diversity was registered during the first experimental days, comprising Lactobacillaceae, Enterobacteriaceae, and Streptococcaceae families, with *Lactobacillus* and *Lactococcus* genera present in all analyzed liver samples. Insect samples presented a greater bacterial diversity during pupae and teneral stages, with Proteobacteria, Actinobacteria, and Bacteroidetes phyla as prevailing taxa, while Firmicutes prevailed the adult and larvae stages communities, with *Lactobacillus*, *Myroides*, *Proteus*, and *Acinetobacter* among the most abundant genera detected.

Overall, these new data on microbial composition and dynamics will strengthen the current knowledge on microbiome characterization from necrophagous insect species, and pathogens transmission by ubiquitous insects.

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**Microbiome, *Lucilia Sericata*, *Salmonella Enterica***



## H14 Bone Microbial Community Succession During Multi-Year Decomposition in an Aquatic Habitat

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**Learning Overview:** After attending this presentation, attendees will better understand how microbial communities colonize bones and shift during long-term decomposition. Attendees will see a microbial community change over the Postmortem Submersion Interval (PMSI). Attendees will recognize that machine learning methods have potential applications to PMSI estimation for aquatic cases with long-term decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by presenting random forest modeling of the postmortem microbiome as a viable PMSI estimation tool for cases with submerged bone evidence.

Microbes are ubiquitous organisms, and the composition of microbial communities are specific to the environments where they reside (e.g., human gut vs. pond water). With advancements in high-throughput sequencing, it is becoming more cost-effective and practical to describe microbial communities with targeted amplicon sequencing (i.e., 16S ribosomal RNA (rRNA) gene amplicon). Characterizing the microbial communities has wide-ranging forensic applications, including answering questions that arise during forensic death investigation such as: when did the decedent die? Identifying the time since death for aquatic cases, known as the PMSI, can be challenging in unattended deaths or cases with concealed bodies. However, the accessibility of high-throughput sequencing technologies allows for microbial evidence to be used in death investigations.

During aquatic decomposition, microbes that colonize carrion or human bodies come from a variety of sources, including the carrion itself, the environment, and insects/scavengers. Bones are subjected to microbial alteration, known as bioerosion, of the bone during decomposition. These bones are a potentially important source of evidence for forensic anthropologists and forensic biologists who routinely extract DNA from bone for decedent identification. Specifically, bone bioerosion by microbes could potentially be used in conjunction with other evidence in cases with longer PMSI estimations (months to years), as PMSI estimation models of microbial community succession on submerged bones have accurately predicted submersion periods.

While microbial models for PMSI estimation are not yet accepted in court, research suggests these models have promise. But, before microbes can be used as reliable evidence, additional studies are needed as knowledge gaps for long-term decomposition in a variety of habitats, such as aquatic ecosystems, exist. Most studies focus on earlier decomposition periods (prior to skeletonization) in terrestrial habitats or sunken remains for short-term (less than one year) decomposition. This study is the first to identify long-term microbial community succession both inside and outside of bones following natural decomposition of carcasses in an aquatic environment to develop PMSI estimation models.

It was hypothesized that the microbial community inside and outside would converge by 18 months of submersion, and that there would be a predictable succession of microbial communities, based on previous success with aquatic decomposition in short-term PMSIs.

To address these hypotheses, replicate human surrogates were submerged (swine [*Sus scrofa domesticus*] carcasses [ $N = 5$ ]) in a natural freshwater aquatic habitat (i.e., pond) to model the PMSI based on the microbial communities that alter the bones' long-term decomposition. Sunken bones were sampled every three months over two calendar years and used targeted gene amplicon high-throughput sequencing (16S rRNA gene amplicon) to describe the microbial communities on (external) and within (internal) the bones. Microbial diversity metrics (alpha- and beta-diversity) varied with microbial community type (internal vs. external), season (spring, summer, winter, fall), and over time (months). Indicator taxa were identified by ANCOM for season, microbial community type, and over time (i.e., Alphaproteobacteria and Bacteroidia). Over time, the "core" microbiome of internal and external communities shifted, but ultimately did not converge to a similar community structure. Random forest models estimating PMSI were accurate (> variation explained > 80%;  $R^2 > 0.95$ ) over this long-term decomposition study.

Overall, the PMSI estimation using microbial communities within and on aquatic bones was a viable tool for application to forensic death investigation. Microbial communities varied over time. Both external and internal microbial communities shifted over time but did not converge to a similar microbial community. Microbial communities could have forensic utility by providing important evidence for unattended death cases, as establishing time since death is essential information for building a case. Moving forward, more studies are needed to expand current knowledge on bone bioerosion as forensic evidence, including additional aquatic environments, longer time points (> 2 years), and with human bones.

### Postmortem Submersion Interval, Microbial Succession, Bone Bioerosion

## H15 Microbes as the Puppet Master: Clear Evidence Microbes Drive the Decomposition Process and Colonization by Insects

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**Learning Overview:** After attending this presentation, attendees will know microbes play a key role in driving decomposition of vertebrate remains and the subsequent attraction and colonization by necrophagous insects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding the mechanisms regulating attraction and colonization of vertebrate remains by blow flies. This information can lead to increased accuracy when estimating forensically important timelines using entomological evidence.

Carrion decomposition typically consists of five stages: fresh, bloated, active decay, advanced decay, and dry. Stages are usually described by microbial and insect activity within and around the remains, and the progression through these stages is thought to be governed by microbial and insect activity in association with other biotic and abiotic factors. Although guided by insect activity, the previously described stages described by Dr. Jerry Payne do not encompass the entirety of the interaction between insects and a resource.<sup>1</sup> The Postmortem Interval (PMI) is defined as the period of time between death and discovery of remains. This is further divided into the pre-colonization interval and post-colonization interval, separated by insect colonization of the remains. Currently, forensic entomologists are capable of calculating the post-colonization interval based on development data available for some necrophagous insects, but the biotic and abiotic factors affecting the pre-colonization interval remain poorly understood and therefore cannot be included in a forensic entomology estimate.

The pre-colonization interval includes the time it takes for insects to detect and locate the resource before colonization. The detection and location phases of the entomological timeline are governed by Volatile Organic Compounds (VOCs) emitted by the remains and associated microbes as a by-product of microbial metabolism. The VOCs produced are responsible for bloat and progression through decomposition and serve as a key mechanism regulating blow fly (Diptera: Calliphoridae) attraction and colonization. However, the degree to which the microbes govern attraction of necrophagous insects is still unknown. Once the factors governing the attraction and location of a resource by necrophagous insects are understood, more accurate forensically relevant estimates can be made.

In the current study, the impact of microbial presence is determined by exposing adult *Cochliomyia macellaria* (Diptera: Calliphoridae) to mouse carcasses with and without microbes in a dual-choice cube olfactometer. Germ-free (axenic) and specific pathogen free (xenic) carcasses were received and preserved overnight at 4°C as preliminary data indicate that freezing a resource alters the attraction of *C. macellaria* to a resource. Axenic and xenic mice were allowed to decompose in a controlled environment to assess the stages of decomposition. A dual-choice cube olfactometer was used to determine preference of adult *C. macellaria* when given the choice of axenic or xenic mouse carcasses based on the VOCs emitted from the treatments. At the conclusion of each trial, all carcasses were exposed to adult flies that were allowed to oviposit on their carcass of choice.

This study determined that the bloat stage of decomposition is absent in axenic mice, as well as later fluid purge. Non-axenic fluid purge occurred on day 6 while axenic fluid purge did not occur until day 13. Olfactometer results indicate that the presence of microbes associated with carrion is a key factor driving attraction of blow flies to carrion at different stages throughout decomposition. In addition, microbial absence reduces *C. macellaria* oviposition by almost 90%.

This study is the first of its kind to completely exclude microbes from the decomposition process and assess the role that microbes play in the progression of decomposition and the attraction of a primary colonizer of carrion. Such data show that microbes are the main driver of attraction and colonization of remains by blow flies and serve as a foundation of exploring microbial variation across decomposing resources in natural settings.

### Reference(s):

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### Forensic Entomology, Necrobiome, Decomposition Ecology

## H16 Investigating Linkages Between Volatile Organic Compounds (VOCs), Total Body Scoring (TBS), and the Stages of Decomposition in Adult Pigs

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of TBS and its connections to the stages of decomposition and VOC production during the decomposition of adult pigs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing more information on pig model decomposition and the use of TBS in combination with the stages of decomposition to provide a more detailed description of decomposition.

Decomposition can be used to assist in the determination of Postmortem Interval (PMI). The complete process of decomposition has traditionally been separated into five stages: fresh, bloat, active decay, advanced decay, and dry remains/skeletonization.<sup>1</sup> Often due to legal reasons, most studies focusing on decomposition use domestic pig cadavers as the closest proxies for human remains. As decomposition does not always occur linearly, determining the stage of decomposition can be difficult; thus, a new method called TBS was proposed by Galloway et al. and refined by Megyesi et al.<sup>2,3</sup> TBS is used to assess the decomposition quantitatively in humans by assigning sections of a body a score based on the progression of decomposition. Since the use of pigs in decomposition research is more prominent than humans, an initial TBS method for pigs was developed by Keough et al. to compare to human decomposition.<sup>4</sup> Previous research suggested dividing the remains into three sections: the head and neck, trunk, and limbs. However, as more significant changes can be seen between the torso and rear than between the trunk and limbs, an amended TBS method was developed for the purposes of this research.

Three pigs (45kg each) were placed in a field at an outdoor research facility in Southern Ontario and were allowed to decompose over three months. Decompositional changes and environmental conditions were recorded. The VOCs associated with the remains were collected and processed using Gas Chromatography/Mass Spectrometry (GC/MS). This project proposed comparing the amended method to the Keough et al. method to determine if it, or the stages of decomposition, better describes the state of the remains. The Keough et al. method was used along with the amended method to calculate the TBS. VOCs associated with decomposition were also compared to the stages of decomposition and TBS to determine if there were any associations.

Quantitative and qualitative analyses showed significant differences between the Keough et al. method and the amended scoring system, particularly between the torso and rear sections of the remains. The analysis of the VOC patterns showed that decomposition VOCs were produced during their characteristic stages of decomposition and occurred linearly with the TBS.

The combination of the amended TBS method and stages of decomposition can be used together to describe the state of the remains better than each method individually. It takes into account the variance of the sections of the body while still describing the state of the remains as a whole.

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### Decomposition, TBS, VOC



## H17 When Primary Colonizers Are Late to the Party: Implications for Using Time of Colonization to Inform Postmortem Interval

Jennifer L. Rhinesmith-Carranza, BS\*, Department of Entomology, College Station, TX 77843; Jeffery K. Tomberlin, PhD, Department of Entomology, College Station, TX 77843-2475

**Learning Overview:** The goal of this presentation is to describe the findings from a behavioral assay conducted with adult *Lucilia sericata* (Diptera: Calliphoridae) blow flies. The results have potential implications for how the entomological pre-colonization interval for remains and its relationship with Time Of Colonization (TOC) estimation assumptions and postmortem interval inferences are interpreted.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting some of the variability that exists in the entomological phases of decomposition, focusing on the pre-colonization interval. Attendees will better understand the assumptions associated with TOC estimations and how variability in the pre-colonization interval may influence how investigators relate TOC and Postmortem Interval (PMI) estimations.

Time of colonization (TOC) estimations calculated by forensic entomologists can be used by investigators when determining a minimum PMI (mPMI) in cases where insect evidence is present. There are a number of assumptions associated with TOC calculations and their relationship to mPMI, including the assumption that insects located and colonized a decedent at the time of death.

The five entomological phases of decomposition can be separated into the pre- and post-colonization intervals. Colonization of remains by insects (e.g., the moment that TOC calculations aim to estimate) marks the start of the post-colonization interval, which includes the consumption and dispersals phases. However, there are three phases that precede colonization (exposure, detection, and acceptance), which make up the pre-colonization interval. This pre-colonization interval and its assumed length are closely tied to the assumptions made regarding insects' rapid location and colonization of available remains.

*Lucilia sericata* (Diptera: Calliphoridae), the common green bottle fly, is a well-studied blow fly species traditionally viewed as a primary colonizer and known for colonizing human and other remains. In this study, behavioral assays were conducted on 7–9-day old *Lucilia sericata* adults in a dual choice olfaction cube using mouse carcasses in the fresh (<24h) and active (72h) stages of decomposition that were either uncolonized or colonized by conspecifics. As a “primary colonizer,” *L. sericata* should be expected to show a preference for fresher carcasses; however, results showed an emphatic olfactory preference for carcasses in later stages of decomposition (93% [mean] preferred late uncolonized carcasses over fresh uncolonized carcasses,  $p < .0001$ ), with existing conspecific colonization having no effect on carcass preference. Furthermore, carcasses in the later stage of decomposition elicited a 37% increase in *L. sericata* foraging behaviors than seen when exposed to fresh carcasses, suggesting that referring to *L. sericata* as a primary colonizer—and the assumptions for pre-colonization interval and TOC estimations that come with the moniker—may be more nuanced than traditionally considered. Considering the preference shown here for carcasses in later stages of decomposition, the assumption of a short pre-colonization interval and its implications for relating TOC and mPMI estimations should be used cautiously. Better understanding the variability associated with the entomological phases of decomposition can help prevent overreaching the use of basic science in applied scenarios.

### Entomology, Time of Colonization, Postmortem Interval



## H18 *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae) Attraction and Colonization Behavior: A Little-Studied Species With Great Forensic Importance

Samantha J. Sawyer, BS\*, Texas A&M University - Entomology, College Station, TX 77842; Jeffery K. Tomberlin, PhD, Department of Entomology, College Station, TX 77843-2475

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how an understudied blow fly species (*Lucilia eximia*) is of forensic importance. This information will enhance the forensic sciences, particularly in case reports involving medicolegal entomology, such as neglect and abuse, as well as will assist in civil litigations involving stored product and urban entomology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the attraction and selection of decomposing animal remains by an understudied blow fly species. This research can aid in forensic entomology case reports in urban, stored product, and/or medical legal entomology.

*Lucilia eximia* (Wiedmann) (Diptera: Calliphoridae) is a forensically important blow fly native to the southern United States and South America and has been documented colonizing both dead and living (via myiasis) animals. Previous research indicated that *L. eximia* will readily colonize small carrion resources, even when colonized with fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). Beyond this, understanding of *L. eximia* biology is largely unknown.

This research aims to better understand carcass selection, acceptance, and survivorship of *L. eximia* on small carrion resources. *L. eximia* was hypothesized to exhibit preferences for select types of carrion for greater offspring fitness. To test this hypothesis, dual choice cube olfactometers were utilized to provide 150 *L. eximia* adults the choice between one of the following: rats and a control (nothing), chicks and a control (nothing), or rats and chicks. Blow flies were allowed eight hours to make a choice between options specified above. This was repeated across three days, allowing the carrion to age without insect access. A second assay allowed carrion access to carrion by placing the carrion within a cage with 150 adult *L. eximia* for eight hours. Eggs were then removed from the carrion and weighed; this study was also conducted across three days to account for carrion aging. A final assay took *L. eximia* larvae and placed first instar larvae directly into a 30cm incision created on the abdomen of chick and rat carrion. Chick carrion had a maggot mass of 50 larvae, while rats had either 50 or 150 larvae placed in the incision site. *Lucilia eximia* were then allowed to reach adulthood before calculating successful pupations, emergence, and adult morphometrics.

Data indicate *L. eximia* does have preference for carrion type and age, specifically, for aged rat carrion, where eggs were laid on aged rat carrion up to two times more than chick. These results provide greater insight to what *L. eximia* are colonizing and where they may be of utmost importance. Specifically, *L. eximia* prefers small carrion and may be of greater significance in medicolegal cases involving neglect of children and small animals (i.e., forensic veterinary medicine). This medicolegal significance is in addition to being important in urban and stored product entomology.

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**Calliphoridae, Medicolegal, Entomology**

## H19 Geographic, Landscape, and Sex Variation in Adult Blow Fly Microbiomes: Implications for Forensics

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**Learning Overview:** After attending this presentation, attendees will better understand how geographic and landscape (i.e., urban and rural) differences affect the microbiomes of adult male and female blow flies (Diptera: Calliphoridae). Attendees will also be introduced to the potential importance of blow fly geographic variation and their associated microbiomes within the broader field of forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the geographic distribution and diversity of forensically relevant blow communities with their nested internal microbiomes. This presentation will provide new data on how adult fly microbiomes vary between sexes captured in three geographic locations of the United States (Appalachia, southeast Alaska, and Mid-Michigan ecoregions), including an analysis of the differences in microbiome structure for blow flies collected in rural versus urban landscapes in Mid-Michigan. Blow flies are forensically important insects used to help estimate a Postmortem Interval (PMI), as they are often the first species to locate and colonize decomposing bodies, including humans.<sup>1</sup> Previous research has shown that microbiomes of flies may affect the succession of insect communities on carrion.<sup>2</sup> The microorganisms influence the decomposition of organic matter by mediating ecological interactions that exist during decomposition.<sup>2</sup>

It was hypothesized (H1) that geographical location and urban versus rural environments of adult blow flies would affect blow fly communities and their internal microbiomes. Blow fly microbiomes are influenced by their environment, and distinct populations of blow flies exist across United States geographic regions, as determined using molecular markers.<sup>3,4</sup> Thus, this variation in genetic populations led to the assumption that blow fly microbiomes could also be distinct based on population, in which ecoregion was used as a proxy for populations. It was further hypothesized (H2) that male and female microbiomes at site-specific locations would vary because the sexes have different behaviors associated with colonizing carrion (e.g., females have extensive contact and lay eggs, but males do not).

To test these hypotheses, blow flies were collected using passive bait traps from nine sites within six ecoregions of the eastern United States (henceforth Appalachia), seven sites near Juneau, AK, and seven sites in Mid-Michigan.<sup>5</sup> Within the Mid-Michigan locations, blow flies were captured along an urban to rural gradient. Urban locations were located in landscapes predominantly developed, whereas rural locations had low development and an abundance of open land.<sup>6</sup> After collection, DNA was extracted using a magnetic bead-based protocol, and the internal fly bacterial communities were identified using high-throughput targeted amplicon sequencing of the 16S gene (V4 region) using methods from published studies.<sup>7-9</sup> Of the blow flies collected in Appalachia, 90.9% were *Phormia regina* (Meigen) ( $n = 11,052$ ) and *P. regina* accounted for 76.9% of the population in rural locations in Mid-Michigan. *Calliphora terraenovae* (Robineau-Desvoidy) was the most abundant species collected in Alaska, accounting for 60% ( $n = 160$ ) of the blow fly communities. *Lucilia sericata* (Meigen) was more abundant (27.4%) in urban areas than rural areas in Mid-Michigan. The internal microbiomes varied among locations and sex of blow flies. From the Appalachia locations, the three predominant phyla detected were Bacteroidetes, Firmicutes, and Proteobacteria. There were significant differences between internal microbiomes of the sexes, with female microbiomes represented by all three phyla and males primarily by Bacteroidetes. The internal microbiomes of female blow flies collected from Alaska consisted predominantly of Proteobacteria and Firmicutes.

Overall, the internal microbial community structures changed depending on the geographical location of adult blow flies. Microbiome differences between male and females also varied significantly. Differences between male and female blow flies resulted in sex-specific microbial indicators, which can be used to further forensic investigations. These data confirm that internal microbiome communities vary between geographic regions and in the future could be used as forensic evidence in criminal investigations. The data could be used to answer questions about the location and environment of the crime scene, as well as estimate PMI. Using microbiomes of forensically relevant blow flies offers ways to improve predicting PMI, which in turn lowers error rates in criminal investigations.<sup>10</sup>

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## **Blow Fly, Microbiome, Geographical Locations**

## H20 Seasonal Differences in Soil Fungal Community Successional Patterns Impacted by Long-Term Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will better understand the joint relationship between soil chemistry and fungal successional patterns occurring during long-term human decomposition, seasonal patterns associated with these changes, and their importance for time-since-death estimations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of long-term and seasonal environmental impacts of human decomposition. This presentation also has implications for Postmortem Interval (PMI) estimates and models.

Decomposition of human remains in environmental settings creates chemically and microbially active hotspots in the soil.<sup>1,2</sup> To date, studies of microbial dynamics in decomposition hotspots have primarily focused on characterizing successional patterns in bacterial communities during the early period of decay, associated with the greatest loss of tissue mass (i.e., active through early advanced decay). However, microbial decomposers involved in the breakdown of organic matter consist of both bacterial and fungal organisms and both are expected to play important roles during decomposition. Additionally, previous studies suggest that soil microbial communities are impacted past advanced decay, highlighting a need for long-term investigations.<sup>2,3</sup>

The goals of this study were to evaluate seasonal patterns in fungal succession and soil chemistry beneath decomposing human subjects for a year and in high resolution. In two seasonal trials ( $n=3$  spring,  $n=3$  winter), donated human subjects were placed on the soil surface at the University of Tennessee Anthropology Research Facility and allowed to decompose naturally for one year. Soils were sampled at two depths: 0–1cm (interface) and 1–15cm (core) across 20 timepoints. Amplicon sequencing was performed targeting the ITS2 region to explore fungal community patterns. Soil pH, electrical conductivity, inorganic nitrogen concentrations, dissolved oxygen, and respiration rates were measured.

Fungal successional patterns were primarily evident at the class taxonomic level. In the spring trial, relative abundances of Saccharomycetes, an Ascomycete yeast, increased between bloat through advanced decay (days 8–72) comprising, on average, approximately 75% of the community in interfaces and 50% in core soils. In the winter trial, Saccharomycetes increased in relative abundance during advanced decay (days 75–110), but with maximum relative abundances of just 25%. Following Saccharomycetes enrichment, Sordariomycetes increased to become the single dominant class, with relative abundances around 25% (spring) and 25%–40% (winter) for the remainder of the study. The Sordariomycetes enrichment was largely due to increases in the orders Hypocreales and Microascales.

Principal Coordinates Analysis (PCoA) shows that fungal community shifts differed significantly by study day and morphological decomposition stage during both seasons (Permutational Multivariate Analysis Of Variance [PERMANOVA],  $p < 0.05$ ). The successional trajectory, as visualized on ordination plots, had two inflection points: (1) the onset of advanced decay (spring, day 16), which corresponded to decreased pH, increased conductivity and respiration rates, maximum ammonium concentrations, and minimum soil oxygen levels ( $38.9 \pm 10\%$ ); and (2) between days 16–86 (spring) corresponding to 85% soil oxygen, decreased ammonium concentrations, and elevated nitrate concentrations.

The observations of fungal community shifts co-occurring with changes in soil oxygenation and inorganic nitrogen speciation show that fungal community and soil chemistry changes are inextricably linked in decomposition hotspots; however, these changes varied by magnitude and timing between seasons.

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### Human Decomposition, Soil Chemistry, Fungal Communities



## H21 The Survival of *Calliphora Vicina* (R.-D.) (Diptera: Calliphoridae), a Forensically Significant Blow Fly, Following Submergence Depends on Life Stage and Submergence Time

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**Learning Overview:** The goal of this presentation is to provide attendees with an overview of blow fly submergence and its importance in forensic entomology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the potential importance of blow flies in aquatic death investigations.

**Introduction:** Forensically significant blow flies (Diptera: Calliphoridae) are commonly used to estimate the post-colonization interval, which can be used to infer the minimum elapsed time since death.<sup>1</sup> In order for forensic entomologists to produce such estimations, they must first estimate the age of the blow fly specimen collected from the field, using existing reference datasets on the average size and developmental progress of the same blow flies species.<sup>2</sup> These reference datasets are often produced locally in a controlled laboratory setting under different conditions commonly experienced in terrestrial environments, while aquatic factors are often not assessed or reported.<sup>2,3</sup> To address this gap in knowledge, this study examined the impact of submergence on the survival of two immature stages of *Calliphora vicina* (R.-D.) (Diptera: Calliphoridae). It was hypothesized that survival rates, based on successful eclosion, will decrease with increasing submergence time for both third instar post-feeding larvae as well as pupae.

**Methods:** A total of 1494 post-feeding third instar *C. vicina* larvae and 1,502 pupae ( $n = 2,996$  total samples) were randomly assigned into control and treatment groups of 30 +/- 2 samples each. The larvae treatment groups were submerged for 1-, 2-, 3-, 4-, or 5-hour time intervals in glass jars with 50mL of distilled water, then transferred to another container of saw dust so they would have adequate conditions to pupariate. The controls were not submerged, and were instead transferred directly to a container of saw dust. Once all the larvae pupariated, they were transferred to a petri dish with filter paper. Similarly, the control pupae were transferred directly to a petri dish with filter paper, whereas the treatment groups were submerged for 24-, 48-, 72-, 96-, or 120-hour time intervals in a weighted cheesecloth pouch to ensure that they remained submerged for the full treatment period. They were then transferred to a petri dish with filter paper. Five replicates of all conditions were performed for each time condition, with control groups for each.

**Results:** Across the 747 larvae specimens assigned to the control group, the mean survival rate at each time period was consistently above the 80% threshold (ranging from an average of 86.0% to a 95.3% survival rate). Larvae in the treatment group revealed a strong negative linear relationship between survivability and hours of submergence. At two hours of submergence, the mean survival was 85.3% but took a drastic decline to 51.7% at three hours. A significant simple linear equation was found ( $F(1,1492) = 885.770, p < 0.000$ ), with an  $R^2$  of 0.373. The larval predicted eclosion rate was equal to  $0.953 + (-0.151)$  hours submerged. The control pupae exhibited similar survivability rates above the 80% threshold, but the treatment group starting from the 24 hours of submergence revealed a lower 57.0 % survival rate, which also showed a strong negative linear relationship; a significance regression equation was found ( $F(1,1500) = 1629.977, p < 0.000$ ), with an  $R^2$  of 0.521. The pupae's predicted eclosion rate is equal to  $0.875 + (-0.008)$  hours submerged. At 96 hours, the survival rate of pupae reached zero.

**Discussion and Conclusion:** Rearing specimens to adulthood plays an important role in helping forensic entomologists identify the blow fly species, which is why factors related to the survivability of specimens are important to consider. These findings show a strong negative linear relationship between submergence periods and survivability of *C. vicina* post-feeding third instar larvae and pupae; submergence was found to be a valuable indicator of successful eclosion. These are forensically significant findings because they highlight the importance of collecting specimens quickly when submergence is suspected to reduce the risk of loss of entomological evidence, as well as aiding in submergence interval estimations.

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### Forensic Entomology, Submergence, *Calliphora Vicina*

## H22 The Effects of Carrion Clothing Color on Blow Fly Oviposition

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the timing of blow fly oviposition in relation to clothing color. Species composition and ambient factors will also be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the timing of blow fly oviposition in relation to clothing color can impact accuracy in estimating the Postmortem Interval (PMI).

Forensic Entomology is the use of insects in medicolegal investigations.<sup>1</sup> The blow fly is typically the first type of insect that will arrive to a carcass.<sup>1</sup> Blow flies are attracted to the volatile chemicals given off by the decomposing carcass and upon arrival will begin to mate and lay eggs on the carcass. Entomologists use blow flies to determine PMI, the time that has elapsed since death. PMI can be affected by many different factors that include temperature, weather, and geographical location.<sup>2</sup> Blow flies are among the most important insects in medicolegal investigations and information about their behavior is important for accurate PMI estimations.

Previous studies examined the effects of clothing on carcass decomposition and patterns of insect succession on clothed and unclothed carcasses. They found that blow flies preferred clothed carcasses over unclothed and would oviposit and stay longer on the clothed carcasses than the unclothed.<sup>3</sup> Another study looked at the color attractiveness of *Calliphora vomitoria*. They determined that blow flies prefer black targets in comparison to white, yellow, red, or blue targets.

In this study, investigators examined the effects of carrion clothing color on blow fly oviposition. Based on the previous studies, it was hypothesized that blow flies would show an oviposition preference to pigs dressed in black. Six fetal pigs, three dressed in white cotton onesies and three dressed in black cotton onesies, were laid out in a grass enclosure for several days until fully decomposed (approximately ten days). Pigs were covered with a layer of chicken wire to prevent interference by scavenging animals. Observations were made twice daily to monitor blow fly oviposition and general insect activity. Maggots in the third instar larval stage were collected from each pig during daily observations. Two trials of six pigs each were conducted in the fall of 2019. Maggot species were then identified using a microscope and taxonomic keys.<sup>4</sup> *T*-tests were conducted to determine if there was a statistical difference between blow fly species present at white- versus black-dressed pigs. There were no statistical differences in species composition between pigs clothed in black garments versus white garments. There were more wasps on pigs dressed in black garments. There was overall more insect activity, including larval and adult blow flies, on pigs dressed in black garments. There was no statistical difference in the number of first, second, or third instar maggots between trials 1 and 2. *P* values for the comparison of instars present in each trial were all larger than 0.05.

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### Forensic Entomology, Clothing Color, Blow Fly

## H23 The Role of Autopsy in COVID-19 Disease

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**Learning Overview:** After attending this presentation, attendees will understand the role of autopsy in COVID-19 disease.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the crucial role of postmortem examination in infectious disease.

The autopsy has played an important role in the study of pathologies. The recent spread of the COVID-19 pandemic is presenting a real challenge for society and health care workers in all sectors. The restrictive measures adopted during the lockdown limited many aspects of personal and family life. The ministerial circulars prohibited the exposure of COVID-19 infected bodies, recommending the immediate closure of the body. Many bodies, at the time of death, were subjected to cremation. Consequently, during the pandemic, many families were unable to see the bodies of their loved ones or carry out funerals, resulting in strong direct and indirect psychological effects. Despite ongoing research, little is known today about the pathological changes of SARS-CoV-2 virus on the body.

During the health emergency, the Forensic Medicine Operating Unit of the “Magna Graecia” University of Catanzaro carried out research aimed at studying the effects of the SARS-CoV-2 virus at autopsy. All deceased patients were examined. The period of hospitalization, medical records, comorbidities, possible complications during hospitalization and cause of death were evaluated for each decedent. All decedents were then subjected to nasal and oropharyngeal swabs. Some of them were also subjected to diagnostic tests and endobronchial swabs.

A total of 20 decedents who were positive for the SARS-CoV-2 virus in life and later died at the Germaneto University Hospital were examined. At the level of the other organs, histiocytosis was noted above all of the spleen with thrombotic areas of the intra-parenchymal vessels.

As part of this study of swabs taken in the postmortem period, some decedents still had a positive Polymerase Chain Reaction (PCR) test for SARS-CoV-2 even 24 hours after death in nasopharyngeal and endo-bronchial swabs.

All autopsies showed a hypercoagulation picture with intravascular thrombo-emboli and features of Disseminated Intravascular Coagulation (DIC). All cases also showed lobar pulmonary fibrosis, in particular at the bases with areas of secondary bacterial infections and pulmonary hepatization with abscess formation. The presence of megakaryocytes was noted in capillaries with endo-alveolar hemorrhagic extravasations and scarring areas. The hilar, paraesophageal, and paratracheal pulmonary lymph nodes demonstrated reactive lymphadenopathy. There was an increase in the density of the bile with gelatinization of the same and degeneration of the hepatic parenchyma. Histiocytosis was noted in the spleen. Additional pulmonary pathology included diffuse alveolar rupture, large areas of pulmonary necrosis due to thrombi, some of which demonstrated evidence of recanalization, early fibrosis, fibrous thickening of alveolar septae, foamy macrophages, and focal hyaline membranes

Finally, molecular investigations were launched to understand virus-induced genetic mutations that generated the hypercoagulation and subsequent pulmonary thrombo-embolism or hemorrhages associated with DIC. This information allowed multidisciplinary comparison with the COVID-19 Operating Units of the Germaneto University Hospital, both in relation to the effects of the disease and the effectiveness of therapies being used.

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### Forensic Sciences, Autopsy, COVID-19

## H24 COVID-19 Suspected Deaths Certified by the Florida District 2 Medical Examiner's Office: Causes of Death and Pre-Existing Medical Conditions

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**Learning Overview:** The goal of this presentation is to disseminate information regarding the causes of death and the most common pre-existing medical conditions identified in COVID-19 suspected deaths certified by the Florida District 2 Medical Examiner's Office.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the body of knowledge regarding the pre-existing medical conditions that may convey an increased risk of death from a SARS-CoV-2 infection.

**Background:** COVID-19 is a highly contagious, predominately respiratory illness caused by the coronavirus SARS-CoV-2. Symptoms range from asymptomatic or mild to severe, with higher risk for complications or death in older adults and people of all ages with certain underlying medical conditions. After report of the first case in Wuhan, China, in December 2019, the first death followed quickly in January 2020. COVID-19 was classified as a global pandemic in March 2020. More than one million global deaths, and more than 210,000 United States deaths, have been attributed to COVID-19. In Florida, all deaths in which COVID-19 is suspected to have contributed must be certified by the district Medical Examiner's Office (MEO) in which the death occurred. Starting with the first such death in this district, all COVID-19 suspected deaths were certified after an investigation that included a review of available medical records. In addition, for each death, a Disaster Death Report (DDR) was prepared by the certifying board-certified forensic pathologist, which included demographic information, a summary of the medical/social history, the cause(s) of death, contributing conditions, and manner of death.

**Methods:** The DDRs for all COVID-19-suspected fatalities certified by the district MEO between March 17 and August 17, 2020, were reviewed. Reported demographic information (age, race, and sex), manner of death, Immediate and Underlying Causes Of Death (ICOD and UCOD, respectively), contributing conditions, and pre-existing medical conditions were tabulated. Results were analyzed for prevalence and tallied (reported as nearest 1%).

**Results:** Of 117 COVID-suspected deaths investigated, COVID-19 was certified as having contributed to death in 114 (97%). Among the COVID-19-related deaths, there were 60% males and 40% females. Ages ranged from 19 to 105 years (mean—68 years, 7 months; median—70 years). The racial distribution was 51% Caucasian/White, 42% African American/Black, and 7% Hispanic/Latino. Prior to infection with SARS-CoV-2, 37% were residents and 3% were workers in a group living setting. Manner of death was Natural in 99%. COVID-19 was the ICOD ( $N=4$ ) or UCOD ( $N=104$ ) in 95% of cases and a contributory cause of death in 5%. COVID-19 syndrome or complications of COVID-19 was the ICOD in 4% (no UCOD specified). In the remaining 96% which designated COVID-19 as the UCOD, pulmonary conditions were overwhelmingly certified as the ICOD. In 76% of cases, respiratory failure was the ICOD and COVID-19 the UCOD, with 53% of these cases attributing respiratory failure to pneumonia or pneumonitis and 1% attributing it to Congestive Heart Failure (CHF) exacerbation. Other pulmonary ICODs included pneumonia (3%), acute respiratory distress syndrome (2%), respiratory infection (1%), and sepsis due to pneumonia (1%). Multisystem disorders included multisystem organ failure (2%) and inflammatory response (1%). Coagulation-related ICODs included intracerebellar hemorrhage, retroperitoneal hematoma, hemorrhagic complications, and acute cerebral stroke (each 1%). Cardiac ICODs (each 1%) consisted of acute myocardial infarction and sudden cardiac death (with CHF the UCOD and COVID-19 contributory). Failure to Thrive was the ICOD in 8% with UCODs of COVID-19 (44%), multiple comorbidities (33%), and metastatic melanoma (11%). The most prevalent contributing and pre-existing medical conditions in COVID-19-related deaths included hypertension (80%), diabetes mellitus (52%), obesity (32%), lipid disorders (31%), dementia (18%), chronic obstructive pulmonary disease (18%), atrial fibrillation (18%), coronary atherosclerosis (17%), CHF (16%), chronic kidney disease (16%), thyroid disorders (11%), chronic anemia (9%), previous strokes (9%), gastroesophageal reflux disease (9%), obstructive sleep apnea (8%), previous cancer (7%), urinary tract infection (6%), tobacco use (5%), dysphagia (5%), and schizophrenia (5%). An additional 45 pre-existing medical conditions with lower prevalence were also identified.

**Conclusion:** COVID-19-related deaths in this district show predominately respiratory causes of death in older adults. Pre-existing medical conditions were common in this cohort, with the most prevalent including hypertension, diabetes, obesity, and hyperlipidemia; however, numerous additional pulmonary, cardiac, neurocognitive, endocrine, renal, neoplastic, and other disorders were also identified.

### COVID-19, Cause of Death, Pre-Existing Medical Conditions



## **H25 A SARS-CoV-2 (COVID-19) Experience at an Academic Medical Examiner's (ME's) Office**

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**WITHDRAWN**



## H26 A Retrospective Analysis of Deaths in Harris County, Texas, for the Detection of COVID-19 Prior to March 2020

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**Learning Overview:** After attending this presentation, attendees will be able to see how a large medical examiner office performed retrospective reviews of autopsy material for the diagnosis of COVID-19 prior to widespread knowledge.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of COVID-19 prior to widely available Polymerase Chain Reaction (PCR) testing and awareness of previously undetected community spread.

The emergence of SARS-CoV-2 rapidly introduced the world to a novel virus that impacted nearly all aspects of society. Coroner and medical examiner offices were required to rapidly adapt what little that was known about COVID-19 into day-to-day death investigations. Case jurisdiction, scene response, and decedent examination were three of the primary areas that were impacted by COVID-19 and required new interview questions, investigative thought processes, and new precautions. Viral PCR testing for SARS-CoV2 was only developed/made available in Harris County in early March 2020. While new operations were quickly and efficiently put into action for incoming cases as of mid-March of 2020, it is believed that SARS-CoV2 had active community spread prior to March 2020. The symptoms of COVID-19 mimic those of other upper respiratory diseases such as influenza, pneumonia, and gastrointestinal illnesses. So, without awareness of COVID-19, it was possible that a COVID-19-related death could have been missed.

A retrospective case study was performed, which looked at all medicolegal cases from January 1, 2020, to March 23, 2020; There were a total of 1,075 cases from January 1, 2020, to March 23, 2020, that were examined at Harris County Institute of Forensic Sciences (HCIFS). The cause and manner of death for these cases were reviewed. Of the 1,075 cases, multiple cases were eliminated based on an elimination criteria of Manner of Death classified as Homicide, Suicide, or Accident-Motor Vehicle Accident (MVA). Only cases with a Natural or Undetermined manner were further reviewed. The narrative of the HCIFS Investigative Report was assessed and cases where the decedents exhibited Influenza-Like Illness (ILI) symptoms such as fever, cough, nausea, vomiting, etc. leading up to their demise were selected for further evaluation. Cases that were signed out with diseases that appeared similar to COVID-19 (influenza, pneumonia, etc.) were also flagged for review. After these two rounds of elimination, 56 cases were determined to be in need of further investigation. The autopsy reports and histology for all 56 cases are being reviewed, and, if necessary, histologic paraffin blocks will be sent out for viral RNA extraction and testing to detect SARS-CoV2. It is believed that this will help determine whether COVID-19 was actively spreading in the community prior to the ability to test for it using nasopharyngeal swabs.

### COVID-19, Retrospective Review, Autopsy Findings



## H27 The Identification of Unrecognized COVID-19 Deaths by Nasopharyngeal Swabs at Funeral Homes

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**Learning Overview:** The goal of this presentation is to demonstrate the role of the medical examiner to diagnose deaths due to Novel Coronavirus (COVID-19) Respiratory Infection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the ability of the medical examiner to identify previously undiagnosed COVID-19 deaths and the benefits of such testing to contact tracing and mortality surveillance, in collaboration with public health partners.

Medical examiner/coroner jurisdictions are required to investigate deaths that pose a threat to public health and safety. Historically, medical examiners have identified and/or monitored and recorded deaths due to a variety of novel infectious outbreaks, including Hanta virus, West Nile virus, and novel H1N1 viral infections. Novel Coronavirus (COVID-19) respiratory infection has caused over 4,500 deaths in Connecticut from March 17 to October 1, 2020. Most deaths occurred at hospitals (53%) and were readily diagnosed and properly certified. COVID-19 deaths that occurred at skilled nursing facilities (42%) and residences (3%) were more of a challenge to diagnose accurately due to the unavailability of viral testing and/or confounding medical illnesses.

The Connecticut Office of the Chief Medical Examiner (OCME) is a single-facility, centralized, state-wide medical examiner system serving a population of approximately 3.6 million people with 31,000 deaths per year. The OCME investigates all sudden, suspicious, and unexpected deaths, which includes threats to public health and safety, as well as deaths in which the remains will be cremated. Approximately 70% of all deaths in Connecticut are investigated by the OCME in some manner. Deaths reported to the OCME from March 17 to October 2020, in which COVID-19 infection was suspected but not previously diagnosed, were triaged to receive a postmortem nasopharyngeal swab at the funeral home.

The investigative staff of the OCME performed 175 postmortem, nasopharyngeal swabs at funeral homes to test for COVID-19. Of these 175 decedents, 110 had SARS-Cov-2 target sequences unique to the coronavirus disease 2019 (COVID-19) detected using the Real-Time JAX COVID-19 RT-PCR assay (Jackson Laboratory, Farmington, CT). Results were generally available within six hours of receipt by the laboratory. The locations of pronouncement of death of these 110 deaths were: skilled nursing facility 61 (56%), private residence 35 (32%), hospital 7 (6%), assisted living home 6 (5%), and hospice 1 (1%).

Of these 110 COVID-19 deaths, 47 initially had been certified as non-COVID-19 deaths and 34 initially were certified as “suspected,” “exposure to,” “possible,” or “rule out” COVID-19. Of these 81 deaths, 64 occurred at skilled nursing facilities (59) and assisted living homes (5). Of the 175 deaths, 46 were reported to the OCME by funeral directors, of which 41 had positive COVID-19 swab results. Of these 41, all were from skilled nursing homes (36) or assisted living homes (5), and 37 had been diagnosed as COVID-19 deaths by OCME investigations triggered by cremation requests from funeral directors. The remaining four were reported at the request of the next-of-kin. There were 26 positive deaths that occurred at private residences and all were reported to the OCME by the local police department. The OCME issued 107 death certificates for these 110 decedents. Three deaths were certified appropriately due to COVID-19; however, the death certification was received after the OCME had started the investigation.

There are several benefits to diagnosing and confirming these COVID-19 deaths. First, it provides essential information to the caregivers, families, roommates, and first responders who have been in contact with the infected patient, resulting in appropriate self-quarantine and containment measures. Next, it provides information at the local and state level to public health partners for contact tracing activities. Finally, and importantly, it improves the accuracy of death certification during a pandemic, ensuring that mortality data has solid value and applicability from the town to the state to the nation and the world. Many of these deaths were identified through the cremation review process and involved deaths at skilled nursing facilities. This initial reporting and surveillance deficiency may be improved by having skilled nursing facilities report all deaths to medical examiners/coroners. This additional quality assurance measure may also help to recognize subsequent outbreaks of infectious diseases at these facilities more quickly. Maintaining open and professional communications with funeral directors is vital as they are valuable colleagues and important surveillance partners. Obtaining nasopharyngeal swabs before embalming is ideal, and, therefore, the timeliness of identifying the suspected case and notifying the funeral director to delay embalming is key.

### Coronavirus (SARS-CoV-2, COVID-19), Nasopharyngeal Swabs, Death

## H28 A COVID-19 Profile in Mumbai City, India, With a Humanitarian Forensic Aspect

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**Learning Overview:** After attending this presentation, attendees will understand the profile of COVID-19 in Mumbai City in a highly populated city. Attendees will also learn a forensic humanitarian aspect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to implement effective management in COVID-19 in a highly populated city like Mumbai. This presentation will also impact the forensic science community by providing dead body management in COVID-19 and implementing the preventive measures in COVID-19 body disposal in either a cosmopolitan city or developing country.

On December 31, 2019, the China Health Authority alerted the World Health Organization (WHO) to several cases of pneumonia of unknown etiology in Wuhan City in Hubei Province in central China. The cases had been reported since December 8, 2019, and many patients worked at, or lived around, the local Huanan Seafood Wholesale Market, although other early cases had no exposure to this market. On January 7, a novel coronavirus, originally abbreviated as 2019-nCoV by WHO, was identified from the throat swab sample of a patient. This pathogen was later renamed as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group, and the disease was named Coronavirus Disease 2019 (COVID-19) by the WHO. As of January 30, 7,736 confirmed and 12,167 suspected cases had been reported in China and 82 confirmed cases had been detected in 18 other countries. On the same day, WHO declared the SARS-CoV-2 outbreak as a Public Health Emergency of International Concern (PHEIC).

The number of confirmed cases worldwide as of October 7, 2020, are 35,659,007, with over 1,044,269 deaths as per WHO COVID -19 Dashboard. The largest number of cases have been reported in the United States with over 7,380,326 cases and 208,787 deaths followed by India with 6,757,131 cases and 104,555 deaths, and Brazil with 4,927,235 cases and 146,675. This pandemic has led to worldwide lockdown, strangling of the global economy, and devastation of human life. Scientists and health care workers are working all over the globe for a possible cure for this deadly virus.

In Mumbai city, there are a total of 214,445 positive cases with 9,105 deaths, with a fatality rate of 4.25% as of October 7, 2020. Among total deaths in Mumbai city, 7,706 deaths are in decedents aged over 50 years. Total tests conducted (October 7, 2020) was 1,178,111 in Mumbai city with 18.20% positivity (October 7, 2020). Contact tracing was successfully done in 31 lakhs; among them, 12 lakhs were high risk and 19 lakhs were low risk. The “chase the virus” policy of Municipal Corporation of Greater Mumbai city in slums resulted in very good control of the virus in highly populated cities like Mumbai. High-risk contacts sent to institutional facilities totaled 123,409 in “chase the virus” policy. Males outnumber the females in positive cases in Mumbai city.

Mumbai city organized well the disposal of COVID-19 human remains, by proper planning and management, including standard precautions by health care workers while handling confirmed COVID-19 positive remains. Challenges included dealing with a diverse population with diverse religious beliefs and delays in disposing of remains in the mortuary due to relatives being under quarantine or due to lockdown restrictions.

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### COVID-19, Mumbai City, Humanitarian Forensic





## H29 Suicide Analysis in the Time of COVID-19 in Orange County, California

*Kelly Keyes, BS\*, Orange County Sheriff Coroner, Coroner Division, Santa Ana, CA 92703*

**Learning Overview:** After attending this presentation, attendees will understand how the COVID-19 (also called SARS-CoV-19 or Coronavirus disease 2019) pandemic affected suicide rates at a large coroner's office, specifically Orange County, CA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting findings analyzing death by suicide in the jurisdiction during the COVID-19 pandemic and examine what role, if any, the pandemic played in people dying by suicide during the six months of the most restrictive lockdown that affected California beginning in March 2020.

There has been much publicity about the effects of the pandemic and the lockdown on the population and mental health.<sup>1,2</sup> Unfortunately, at this point in the crisis, any long-term effects on suicide rates and mental health cannot be fully understood, including financial stressors and future potential lockdowns; however, the information on those dying by suicide in this initial phase of the pandemic lockdown is available.

This presentation aims to examine one of the many indirect consequences of the pandemic on death rates at a medicolegal death investigation office, one that in addition to the burden of increased deaths directly attributed to the virus, adds significantly to the burdens on staff at all levels of the office along with other indirect pandemic deaths (such as accidental overdoses). This presentation will also briefly address other classifications of death during this time frame.

This presentation will include comparisons to suicide rates from the five prior years and will include analysis of investigation reports to determine if there was any direct mention of the pandemic by the victims, either to informants or via suicide note or other writings, as well as reviewing interviews with friends and family to see if they attributed the pandemic as a possible explanation of suicide, as well as any other factors mentioned. Analysis will be done on various age groups, races, and genders, and will also look at past medical history, marital status, and living situations. This presentation will examine the methods through which people are taking their lives in this timeframe as compared to the prior years and will also address toxicology findings.

This presentation will impact the forensic science community by serving as a guideline for potential future resource allocation in times of pandemic, as well as serve a public health role for future evaluation to save lives should the nation again be faced with such challenges. This presentation can also be used by medicolegal death investigators to have heightened awareness of suicide rates during a pandemic.

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### Suicide, COVID-19, Medicolegal Death Investigation

## H30 Histological Parameters of Myocarditis in Relation to the Cause of Death in Clinical and Forensic Autopsy Pathology

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**Learning Overview:** The goal of this presentation is to provide insight in the histological spectrum of myocardial inflammation and myocarditis and its relation to the cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information that may aid practitioners in interpreting myocardial inflammation and its relation to the cause of death.

Myocarditis is a common histologic finding at autopsy. However, the significance of a histopathological diagnosis of myocarditis can be a matter of debate, even more so since the clinical presentation of myocarditis is highly variable and may range from subclinical symptoms to sudden arrhythmic death. Interpreting the role of myocarditis as causal, contributory, or of no significance at all in the cause of death can be especially challenging in cases where other, non-conclusive pathologic and/or medicolegal findings are also present.

To further evaluate the significance of myocarditis as a cause of death, a retrospective double-center study of forensic and clinical autopsy cases over a five-year period was performed. Specifically, the spectrum of histological inflammatory parameters in the myocardium in an autopsy population of 89 adult cases was reviewed and subsequently related these to the reported cause of death. Myocardial slides were reviewed for the distribution and intensity of inflammatory cell infiltrates; the predominant type of inflammatory cells; and the presence of fibrosis, hemorrhage, edema, and inflammation-associated myocyte death. Next, the cases were divided into three groups based on the reported cause of death at autopsy. Group 1 ( $n=27$ ) consisted of all individuals with an obvious unnatural, non-myocarditis-related cause of death. Group 2 ( $n=29$ ) included all individuals in which myocarditis was interpreted to be one out of more possible causes of death. Group 3 ( $n=23$ ) consisted of all individuals in which myocarditis was interpreted as the only significant finding at autopsy and no other cause of death was found.

Systematic application of histological parameters in these three groups showed that only a diffuse increase of inflammatory cells could discriminate significantly between an incidental presence of inflammation (Group 1) or a potentially significant one (Groups 2 and 3). Still, in 21 cases of Group 1, myocarditis could be diagnosed. Ten of these cases constituted multifocal myocarditis, and one showed a diffuse type of myocarditis. This indicates that myocarditis can be an incidental finding not related to the cause of death. Significant differences in inflammatory parameters were not found between Groups 2 and 3. In conclusion, caution should be taken with definitive statements pertaining to the cause of death due to myocarditis in order to prevent a potential underestimation of other potential causes of death, such as familial arrhythmias or unnatural causes of death.

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### Myocarditis, Histology, Cause of Death

## H31 Evaluating Small Vessel Neutrophils as a Marker for Systemic Infection

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**Learning Overview:** After attending this presentation, attendees will be able to determine how an increase in the number of neutrophils in small-caliber vessels of organs can be used to determine whether sepsis or severe systemic infection was present at death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simple autopsy scoring system for the sepsis.

Accurate autopsy identification of sepsis requires integration of clinical information, antemortem laboratory studies, and gross and histological findings. Studies have investigated sepsis-related deaths through the use of immunohistochemical biomarkers, including those that are upregulated in inflammation, such as selectins, integrins, Vascular Endothelial Growth Factor (VEGF), and Intercellular Adhesion Molecules (ICAM1). These studies used small sample sizes and the use of immunohistochemistry markers is not routine. Additional research has looked at quantifying neutrophils in acute splenitis, but that study did not find a significant correlation. The objective of this study was to determine, through the use of a scoring system, whether an increase in the number of neutrophils in small-caliber vessels of the liver, heart, and lungs can be used to predict if death was due to sepsis or severe systemic infection.

A retrospective case control study over an eight-year period was performed. An electronic information system was used to identify adults who died of either sepsis or systemic infection. Adults who died suddenly of heart disease in the same year were used as controls. Cases were excluded if there was severe decomposition, if the decedent was less than 18 years old, or if histology was unavailable. Data extracted included demographics of the decedent, cause of death, presence of conditions that could interfere with an inflammatory response, history of hospitalization, and results of microbiology cultures. Histological sections of the liver, heart, and lungs for cases and controls were assessed by two doctors who blinded to the clinical information and cause of death. Organs were scored for neutrophilic inflammation based upon a predetermined grading system. Scores of 0, 1, and 2 were assigned according to mild, moderate, and florid neutrophilic presence, respectively; a total score was also assigned based on the sum of the scores from all three organs. Comparison between the cases and controls were made using the student's *t*-test for continuous variables and chi-square for proportions and categorical variables. A test for trend looking at the percentage of sepsis cases as the scoring system increased was assessed using simple linear regression. Statistical analyses were conducted using Microsoft® Excel® and Minitab® 19.

Two hundred three cases met the inclusion criteria; they consisted of 100 sepsis cases and 103 controls. The mean age of the total group was 55.71 years, with a range from 18–92 years. Overall, 123 cases were male and 80 were female. Within the sepsis cases, the mean age was 53.1 years, 53 (66.25%) were female, and 47 (38.21%) were male. The controls were slightly older with a mean age of 58.3 years and consisted of 27 (33.75%) females and 76 males (61.79%). A similar proportion of cases (51.52%) and controls (48.48%) had a condition that could interfere with the decedent's response to an infection ( $p=0.656$ ). Comparing the histological grading between cases and controls found a statistical difference with the neutrophil grading in the liver ( $p<0.001$ ), lung ( $p<0.001$ ), heart ( $p<0.001$ ), and between the combined total score ( $p<0.001$ ). Examining the percentage of sepsis cases as the histological neutrophilic score increased found a positive slope in all three organs; however, only the linear regression looking at the lung ( $p=0.03$ ) and the combined score ( $p=0.001$ ) were statistically significant. Despite the above results, it was also noted that in all three organ systems and with the combined score, there were sepsis cases with low scores and controls with moderate and florid neutrophilic infiltrates.

In conclusion, this study showed statistical differences in the number of intravascular neutrophils in deaths due to sepsis in comparison to the control group. Although the deaths from sepsis were not always associated with a florid neutrophilic infiltrate, the scoring system presented can be used as an additional tool in determining the presence of sepsis or severe systemic infection as a mechanism of death.

### Autopsy, Sepsis, Neutrophils

## H32 Non-Skeletal Injuries Related to Cardiopulmonary Resuscitation (CPR): An Autopsy Study

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**Learning Overview:** After attending this presentation, attendees will better understand the range and severity of non-skeletal injuries associated with CPR.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing additional information relating to injuries from CPR and improving the knowledge and tools necessary to differentiate medical intervention-related injuries from other injuries in the forensic autopsy setting.

The current standard technique for CPR, initially described in the early 1960s, has quickly become the expected response for all persons found without a pulse or respiration. External cardiac massage stands as the mainstay of therapy with the goal of producing blood movement, leading to a temporary circulation that achieves adequate oxygenation of terminal vessels by raising intrathoracic pressure, until Return Of Spontaneous Circulation (ROSC) is achieved. In spite of its potentially lifesaving properties, external cardiac massage consists of repeated blunt force trauma to the chest, which can lead to extensive traumatic skeletal injuries. Once the stability of the bony skeleton has been compromised, visceral injuries may also occur. Numerous autopsy-based studies have documented the incidence and patterns of rib and sternal fractures associated with attempted CPR, but there is relatively little data on the incidence and severity of non-skeletal CPR-related injuries. Both skeletal and non-skeletal CPR-related injuries can be difficult to distinguish from traumatic injuries that were present prior to the onset of CPR. Therefore, it is helpful for forensic pathologists to understand the types and severity of non-skeletal injuries that may be associated with CPR.

In order to contribute additional knowledge to this area, 1,878 autopsy reports between the years of 2017 and 2019 were retrospectively searched for documentation of CPR-related injuries. Both hospital and forensic autopsies were included. Among the 1,878 autopsies performed within this time frame, 338 cases had CPR-related injuries documented in the autopsy report. Of these 338 autopsies with either skeletal or non-skeletal CPR-related injury, 28% ( $n=93$ ) had a non-skeletal, visceral injury documented at autopsy. Conversely, 1.5% had visceral injuries without skeletal injury. The average age for non-skeletal injuries was 60.3 years and there was a slight male predominance with a male-to-female ratio of 1.27. The three most common causes of death were cardiovascular diseases (49%), substance abuse (11%), and trauma (8%). In ten cases (10.7%), a LUCAS<sup>®</sup> device had been used to perform resuscitation.

Out of the total number of cases with reported injuries, hemothoraces were present in 9.5% of cases, with most cases having bilateral collections averaging 441ml of blood on the right and 638ml on the left side of the chest (range: 10–3,150ml). Hemopericardium was documented in 2.7% of cases, with an average of 117ml of blood collected during the autopsy (range: 30–200ml).

Visceral contusions were the most common type of non-skeletal CPR-related injury, documented in 21.3% of cases, predominantly involving the heart and lungs, closely followed by contusions of the neck soft tissue and surrounding structures. Sixteen cases (4.7%) had visceral lacerations involving the pericardium ( $n=7$ ), heart ( $n=3$ ), aorta ( $n=1$ ), liver ( $n=4$ ), and esophagus ( $n=1$ ). The esophageal laceration resulted from perforation by a King airway tube. Lung lacerations were not observed.

The goal of this study is to provide additional documentation on the range and severity of various types of resuscitation-related visceral injuries. Based on this study's data, the most common non-skeletal injuries are contusions of the heart, lungs, and soft tissues. Rare injuries include lacerations of the pericardium, heart, aorta, liver, and esophagus.

A limitation of this study is that the number of decedents who received CPR among the 1,878 searched cases could not be determined due to inconsistent documentation of clinical information in the autopsy report. Therefore, the incidence of these injuries resulting from CPR could not be determined.

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### Forensic Autopsy, Cardiopulmonary Resuscitation, CPR

## H33 Extracorporeal Membrane Oxygenation (ECMO) in the Forensic Setting: A Series of 19 Forensic Cases

*Andrew J. Layman, MD\*, Mayo Clinic, Rochester, MN 55905; Peter T. Lin, MD, Mayo Clinic, Rochester, MN 55905*

**Learning Overview:** After attending this presentation, attendees will have be familiar with the ECMO procedure, including common scenarios, indications, configurations, and complications that are likely be to be encountered by forensic pathologists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness among death investigation professionals of a complicated intervention used as salvage therapy in individuals who develop acute cardiorespiratory failure, a population whose deaths are likely to fall under medical examiner jurisdiction.

**Introduction:** ECMO is a surgical intervention employing vascular cannulation and a gas exchange circuit to provide circulatory and respiratory support to critically ill patients with severely compromised cardiopulmonary function. Clinical settings may include both acute (trauma, cardiac arrest) as well as subacute and chronic conditions (idiopathic lung disease, viral pneumonia, sepsis). More recently, ECMO has gained some attention as a potential treatment for severe COVID-19 infections. As a salvage therapy, ECMO is often the last major intervention taken before death and thus presents a unique set of challenges to medical examiners.

This study describes the characteristics of a series of 19 deaths involving patients who were on ECMO at the time of death, including clinical indications, types of circuit configurations (venovenous versus venoarterial), causes and manners of death, gross and histopathologic findings at autopsy, and complications that may arise with both short -and long-term ECMO support.

**Methods:** Medical examiner files (MDILog, ORA, Inc.) of the Southern Minnesota Regional Medical Examiner Office were searched for keywords “ECMO” or “extracorporeal membrane oxygenation” between 2013 and 2019.

**Results:** Nineteen cases of decedents who were on ECMO at the time of death were identified. All 19 cases were autopsied, and all were considered medical examiner cases. The median age was 36 years (range, 2–83 years) with 11 men and 8 women represented. The circumstances surrounding the initial presentation included: sudden death in a young person ( $n=6$ , mean age: 28.7 years), trauma ( $n=3$ ), history of substance abuse ( $n=3$ ), possible or confirmed homicide ( $n=2$ ), therapeutic complication ( $n=2$ ), work-related injury ( $n=1$ ), drowning ( $n=1$ ), and hypothermia due to environmental exposure ( $n=1$ ). Of the two therapeutic complications, one was directly related to the ECMO therapy.

The underlying causes of death included: seven injury-related deaths (including drowning/hypothermia, accidental falls, assault, work-related injury, and overdose); six respiratory-related natural diseases (including viral pneumonia, rapid onset organizing pneumonia, pulmonary thromboembolism, and complications of lung transplantation); and six deaths due to cardiac-related natural diseases (including ischemic heart disease, familial cardiomyopathy, valvular heart disease, and aortic aneurysmal disease).

The time spent on ECMO varied greatly from less than one hour to ten months. The majority of cases ( $n=17$ ) utilized Venoarterial (VA) cannulation and two cases utilized Venovenous (VV) access. The most common cannulation sites for central VA-ECMO were the right atrium and aorta, while peripheral VA most commonly accessed the right femoral artery and right femoral vein. VV-ECMO accessed the right internal jugular and right femoral veins. Complications encountered due to ECMO intervention included cannulation site bleeding, pneumohemopericardium, retroperitoneal hematoma, limb ischemia, clotting, and cannula dislodgement.

**Conclusion:** The patient population likely to receive ECMO has significant overlap with the populations that are likely to be reported to the medical examiner. As ECMO therapy has become increasingly available in the community, it is of importance for medical examiners and death investigators to be familiar with the procedure as well as its limitations. Familiarity with ECMO and its sequelae allows for the proper documentation of postmortem findings and fosters an informed determination of the cause and manner of death.

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### ECMO, Forensic, Cardiovascular

## H34 Postmortem Changes of Female External Genitalia and Their Importance in Suspected Sexual Abuse

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**Learning Overview:** The goal of this presentation is to inform attendees that lividity was not found in female genitals in postmortem examination and any discoloration in this area should be well investigated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that lividity of female genitals was not found in postmortem examination and that before putrefaction sets in, any discoloration should be well investigated to rule out sexual abuse.

Examination of the female external genitalia to assess for sexual abuse is performed in living individuals, and the interpretation of the findings is based on evidence-based studies. However, in the deceased, no such studies are available, and postmortem changes could present as suspicious findings that can be mistaken for trauma. Patches of discoloration in the hymen were reported previously in one case as hypostasis (i.e., livor and lividity), and based on this finding, it was listed as a finding that is not associated with trauma.<sup>1,2</sup>

A retrospective study was conducted at the Center of Forensic and Legal Medicine in Dammam, Saudi Arabia, over a four-year period. The study included 30 deceased women in whom photographic documentation of their external genitalia was assessed for postmortem changes.

The postmortem interval ranged from less than 24 hours to more than 100 days, and the ages of these deceased women were in the 20- to 40-year-old age group. Death was due to pressure over the neck in 35% of the cases, blunt force trauma in 19%, and sharp force injury in 11%. Putrefaction was absent in 87% of cases, mild in 3%, and advanced in 10%. Hypostasis was on the back of the body in the majority of cases (77%). In cases where the hymen, vagina, and/or fossa navicularis were clearly visible, none of these areas showed any hypostatic discoloration. A comparison between antemortem and postmortem appearance of the hymen in one case clearly showed the absence of hypostatic changes in the hymen and besides slight paleness (explained by loss of actively moving blood), the hymen looked exactly like that in the antemortem picture with no distinct discolorations. In conclusion, before putrefaction sets in, any discoloration of the hymen and the area around it should not be attributed to hypostasis. Further study of such an area should be pursued to establish the etiology of the discoloration, and this could include biopsy and histological evaluation of the area of concern.

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### Lividity, Hymen, Sexual Abuse



## H35 Hurricane Harvey: Fatality Overview

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**Learning Overview:** After attending this presentation, attendees will have learned about the storm-related deaths in Harris County, TX, during Hurricane Harvey and the challenges of performing autopsies during a natural disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the deaths encountered in mass flooding, as well as associated circumstances and geography.

In August 2017, Hurricane Harvey made landfall in Texas and over five days dropped an average of 47.4 inches of rain across Harris County, nearly reaching the average annual rainfall for the county. It is estimated that over 600,000 cars and 150,000 structures were flooded, forcing 37,000 people into shelters. Using information collected by forensic investigators, medical examiners, and forensic anthropologists at the Harris County Institute of Forensic Sciences, 37 flood-related deaths between August 26, 2017, and September 15, 2017, were reviewed for trends in decedent demographics, location found, and circumstances of death.

Of the 37 confirmed deaths, 34 were classified as drownings, and the 3 remaining deaths consisted of a natural death and two storm-related accidents. Of the 34 drowning deaths, 25 were male and 9 were female with ages ranging from 6 to 84 years. Of the drowning deaths, five individuals were recovered indoors, and the remaining 29 were found outdoors. Of the decedents recovered outdoors, 14 were found in or near a vehicle (six of these deaths were located in a single van that was swept off a roadway by rising water) and six were found in or near a body of water following two separate boating accidents. Both boating accidents involved volunteers attempting to aid in rescue efforts. Of the individuals recovered indoors, two were found at their place of employment and two were found in their residences.

There were multiple obstacles to forensic evaluation during the natural disaster, including inaccessibility of scenes and decedents during active flooding, movement of decedents by currents, and issues regarding identification of decedents. These issues and the strategies used to navigate them will be discussed.

Planning and construction are underway in Harris County and surrounding counties to improve drainage and flood warning systems based on areas impacted by high-water levels; however, fatality analysis provides a unique approach to identifying high-risk areas and trends. The information gathered above will be compared to Harris County Flood Control District data on nearby bodies of water and their corresponding watershed zones and floodplains to identify fatality risk areas that could aid in city planning and education.

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### Flood, Disaster, Hurricane Harvey

## H36 A Framework for Assessing Mortality and Morbidity After Large-Scale Disasters: The National Academies of Science 2020 Report

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**Learning Overview:** The goals of this presentation are: (1) to compare the utility of mortality “counts” and “population estimates” to assess the impact of disasters, (2) to strengthen existing systems to improve individual-level mortality data, and (3) to implement standardized definitions for death data collection and reporting

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the variability of death certificate data reporting systems in the United States precludes the collection and reporting of accurate death data to measure the impact of a large-scale disaster and impairs the ability to utilize the data for remediation of active disasters and prevention and planning for future events.

The National Academies of Science convened an expert committee in 2019–2020 to assess the difficulties in collecting accurate mortality and morbidity data after large-scale mass disasters and to prepare a report to assist data collectors.<sup>1</sup> The report was initiated in response to the inability to collect and analyze mortality and morbidity resulting from the hurricane that devastated Puerto Rico. Death counts were minimal in the face of multiple catastrophic events, and retrospective epidemiological and population statistical studies produced much larger numbers that were disparate. All panelists agreed that a better framework was needed to assist medicolegal death investigators and health care providers in data collection so that the true impact of a disaster could be measured. Among the report recommendations are several that directly impact medical examiner/coroner systems. They provide information, tools, and standards that can improve death data collection by existing death investigation systems.

Recommendation 2-1 states multiple federal agencies should adopt and support the use of a uniform framework for assessing disaster-related mortality and morbidity before, during, and after a disaster.

Recommendation 2-2 states both individual counts and population estimates should be used as accepted standards for reporting mortality and morbidity by state, local, tribal, and territorial entities and supported by the federal agencies as indicators of mortality and morbidity to determine the impact of disasters over time.

Recommendation 3-1 recommends the Centers for Disease Control and Prevention (CDC) through the National Center for Health Statistics (NCHS) incentivize and strengthen existing death registration systems (medical examiner and coroner systems) to improve the quality of disaster-related mortality data at all levels.

Recommendation 3-2 recommends the NCHS, working with the states, should update the Model State Vital Statistics Act to drive uniformity of data collection with respect to disaster-related mortality. Among specific issues identified were the following. Death certificates for disaster deaths do not mention the disaster so they are not coded as disaster-related and may not be counted. Recognizing that there is no specific box on the death certificate asking if the death was disaster-related, the committee recommended a drop-down box that would collect the disaster information in question. Deaths may not be attributed to a disaster by some systems unless due to the actual immediate forces of the disaster when in fact a death may be disaster-related due to unsafe or unhealthy conditions while preparing, responding to, or during recovery. Partially attributable deaths and morbidity (i.e., where the disaster more likely than not contributed to the death) are not identified and not counted. Discussions of the above led to the adoption of the CDC Terminology for attribution of deaths as Direct, Indirect, and Partially attributable death as a uniform framework for reporting mortality and morbidity.

The committee recognized the importance of professional training and support with Recommendation 3-4 tasking the CDC with state agencies and professional associations, (National Association of Medical Examiners [NAME], International Association of Coroners and Medical Examiners [IACME], American Academy of Forensic Sciences [AAFS]) to strengthen the value, capacity, and capability of the medicolegal death investigation system to improve investigation, training, data development and collection, and case management.

Recommendation 3-5 recommended that state, local, tribal, and territorial public health and emergency management departments should integrate the professionals and agencies from the medicolegal death investigation and death registration systems in all aspects of preparedness and planning. This should involve the consideration of moving mortality management out of Emergency Support Function #8 (ESF8) and creating a separate ESF dedicated to mortality management to focus attention on assessing mortality during and after disasters.

The committee also recognized and discussed recommendations supporting the utility of population-level estimates of mortality and morbidity, especially excess mortality, as a true measure of the wider impact of a disaster and as an important element in preparation, mitigation, and pre-planning for future disasters.

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### Mass Disasters, Disaster Mortality, Disaster Statistics



## H37 Excited Delirium Unassociated With Police Involvement

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**Learning Overview:** The goal of this presentation is to educate attendees about excited delirium syndrome and present examples of excited delirium where police have not been involved.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing the ability of attendees to understand the diagnosis of excited delirium syndrome.

In the wake of several high-profile deaths associated with police involvement, several articles have been published in the lay press questioning the existence of the diagnosis of excited delirium syndrome.<sup>1-5</sup> These articles imply that the diagnosis has been used in lieu of associating a death with police involvement. In other words, as a way of covering up a death that has been caused by "excessive force" on the part of police officers. It is also implied by these articles that in the absence of police involvement, the diagnosis of excited delirium syndrome does not exist. The purpose of this presentation is to present several cases of excited delirium without police involvement that have presented to the Erie County Medical Examiner's Office over the past five years.

Although rare overall, in forensic pathology, it is not uncommon to see deaths that are related to premortem or perimortem behavior consistent with the clinical diagnosis of excited delirium syndrome.<sup>6</sup> Often the decedent's behavior is associated with an underlying natural disease or an intoxication that has caused the decedent to exhibit bizarre or psychotic behavior.<sup>7,8</sup> While in the classic clinical definition of excited delirium syndrome, no definitive cause of death is identified, per research, deaths have been ascribed to unnatural causes, such as injuries that the decedent has sustained, the decedent's underlying natural disease in the setting of an agitated state, or the direct effect of the disease or intoxicant on the decedent's body. In certain cases, the decedent's bizarre behavior has been witnessed by one or several individuals, while in other cases the only clue to the decedent's premortem or perimortem behavior is the state of their surroundings.

A review of files identified four deaths associated with decedent behavior consistent with the clinical diagnosis of excited delirium syndrome. In two of these cases, the decedent was witnessed by multiple people to exhibit psychotic behavior in the premortem and/or perimortem period. In the other two cases, the decedent was discovered with injuries or in surroundings that demonstrated that the decedent was exhibiting psychotic behavior before their death, such as destroying furniture or punching walls. The decedents were pronounced dead in the hospital in the two former cases where their behavior was witnessed, and the decedents were discovered dead at the scene in the latter two cases. Of note, in one of the cases where the decedent was discovered dead, there was a history of previous incidents of psychotic behavior. In two of the cases, the psychotic behavior was associated with an acute viral encephalitis, one case was associated with dementia pugilistica, and one case was associated with an acute cocaine intoxication.

Excited delirium syndrome is a condition characterized by psychomotor agitation and confusion, often associated with psychotic behavior and death.<sup>8</sup> Further investigation often reveals that the person in question has an underlying natural disease or an acute drug intoxication that may explain their behavior. Although in some well-publicized instances there has been police involvement due to the behavior of the persons involved, this is not invariably the case. The purpose of this presentation is to document cases where decedents have exhibited symptoms consistent with the diagnosis of excited delirium syndrome and deaths have occurred in the absence of police involvement.

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2. Meyer, Matthew. Police Call it "Excited Delirium." Civil Rights Groups Call it a Sham. *Harvard Civil Rights – Civil Liberties Law Review*, November 15, 2019. <https://harvardcrcl.org/police-call-it-excited-delirium-civil-rights-groups-call-it-a-sham/>.
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### Excited Delirium, Law Enforcement, Psychosis

## H38 Paschen's Law and Electrical Burns

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**Learning Overview:** The goal of this presentation is to acquaint attendees with Paschen's Law and what effect it has on the development of high-voltage electrical burns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assisting attendees in being able to better reconstruct high-voltage electrical deaths.

The paper by Wright and Davis that was published by the *Journal of Forensic Sciences* in 1980 (The Investigation of Electrical Deaths: A Report of 220 Fatalities) is one of the seminal papers that the forensic pathologist refers to when investigating a possible death by electrical means.<sup>1</sup> In their paper, the authors show that about 95% of persons who have become engaged with high voltage have some type of thermal lesion present.

Further to the point, the authors state (regarding high voltage) that "even momentary contact is associated with burning."<sup>1</sup> This statement is invariably true. What the authors fail to realize or explain is that burning does not at all require a continuous metallic or semi-conducting current path for a burn to occur. In other words, there is another mechanism that creates thermal lesions other than that of ohmic heating from electrical current flow through the cutaneous surfaces.

The laws of electrical engineering include a law developed by Friedrich Paschen in 1889. The law describes the size (distance) in differing gaseous media for the gas (air) to ionize and begin to conduct electrical current. In normal air, and with voltages of about 7,500 volts RMS (a common power line voltage), electrical current can "jump" or arc a distance of about 35cm (14"). During the arcing process, temperatures of between 5,000°F and 10,000°F are developed, with much of the thermal injury creating thermal burns on the skin in the event a human body is so exposed.

Testing by this study on freshly harvested porcine legs (ethically obtained from a meat market) demonstrate the arcing phenomenon. This study used various sized high-voltage transformers electrically powering a metal grid (electrode) separated from an oppositely charged porcine leg. After power was applied, burning was seen to occur even though an air gap existed between the grid (electrode) and the porcine flesh. Moreover, using current-limited transformers and open-shutter photography, the current path through open air can be seen to move, creating a pattern that does not necessarily match the shape of the electrode that is being excited.

This research demonstrates that electrical burns from high-voltage source energy do not require that there be direct electrode-to-skin contact for burns to occur. For purposes of this presentation, the remote presentation will allow for actual live testing to take place from the lab, demonstrating the veracity of the material being presented. Different dwell (exposure) times will be presented, showing the development of burns almost instantaneously, as well as the propensity of the ionized path to "wander," creating a pattern that does not always mirror the shape of the electrode.

Finally, the Wright/Davis paper made attempts at explaining the difference between *power* and *energy* when analyzing electrical burns.<sup>1</sup> The present study will explain the differences between the two and will explain the corrections necessary to make the prior paper accurate in terms of thermodynamics and heat transfer.

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### Electrocution, Arc, Burn

## H39 Electrothermal Fatalities

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**Learning Overview:** The goal of this presentation is to broaden insight and understanding of the relevant pathophysiology and pathology as well as the setting and manner of death associated with electrothermal fatalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how South Africa has an exceptionally high rate of electrothermal fatalities. These case studies serve to illustrate the spectrum of pathology, circumstance, and settings which may be relevant, with particular reference to manner of death such as homicide, accident, and suicide.

After attending this presentation, attendees will have a better understanding of electrothermal injuries and fatalities, more specifically in the setting of a developing country where there are relatively low levels of education, substandard housing, and lack of physical infrastructure. The very high incidence of fatal electrothermal injuries in a large metropolitan community will be demonstrated through a variety of case vignettes. Forensic pathologists and death investigators in particular should at all times be mindful of the possibility that such deaths may be of an accidental, suicidal, or homicidal nature.

Pretoria is the executive and administrative capital of South Africa, with a population of approximately three million people. A relatively large proportion of the inhabitants live in subeconomic housing units and informal settlements (including many so-called “squatter camps”), where illegal and uncertified electrical connections are prevalent. The Pretoria Forensic Pathology Service (medical examiner office) on average admits more than 2,000 non-natural fatalities for investigation annually, including a relatively large number of fatalities associated with exposure to electricity. The number of electrocutions in all forms has increased substantially over the past decade, but the reasons for this are not well studied or reported on, and the circumstances under which such fatalities occur are very divergent (including exposure to domestic current, high-voltage incidents, and lightning strikes).

This presentation aims to provide information regarding the various settings which are associated with electrocutions in this region, also providing an overview of the pattern(s) of injury as well as the morphology of the typical—and indeed almost unique—injuries often associated with such events. The presentation will in particular attempt to demonstrate that the admissions to the Pretoria Forensic Pathology Service represent widely divergent circumstances and that the attending pathologist and/or death investigator should be very attentive and informed in this regard, as eventual determination of the probable mechanism as well as manner of death may greatly depend on autopsy and scene findings. The case presentations will include cases of accidental manner of death (including mother and child electrocution), self-inflicted/suicidal exposure to domestic current, fatal high-voltage electrocution during attempted theft of pylon cables, and electrocution of a victim during a rape attack. Graphic images of the scene where such incidents occurred as well as the relevant autopsy findings will be shared, in conjunction with a discussion of the basic physical parameters associated with such electrocutions. In addition, a brief discussion of the classification of manner of death relevant to these cases will be presented.

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### Electrocution, Electrothermal Injuries, Manner of Death

## H40 Fatalities Due to Major Vascular Trocar Injuries in Laparoscopic Surgery

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**Learning Overview:** After attending this presentation, attendees will understand the epidemiology, risk factors, and autopsy findings associated with major vascular trocar injuries in laparoscopic surgery during the past 16 years in New York City, NY, and Houston, TX.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying risk factors and discussing autopsy findings associated with major vascular trocar injuries in laparoscopic surgery, with the aim of reducing these deaths.

In the United States, approximately two million laparoscopic operative procedures are performed every year.<sup>1</sup> The incidence of complications is as high as six percent for major procedures.<sup>2</sup> More than half of the associated serious injuries and deaths occur during the first step of the operation, when a trocar is used to gain entry into the peritoneal cavity to create a pneumoperitoneum.<sup>3,4</sup> The most common injury is to vascular structures, even when shielded or optical trocars are used, and the aorta is the most common vascular injury in fatal cases.<sup>1</sup> The estimated incidence of major vascular injuries is 0.05%, with a resulting mortality of up to 17%.<sup>5</sup>

The Office of Chief Medical Examiner of the City of New York and the Harris County Institute of Forensic Sciences electronic databases were searched for all fatalities where the cause of death was associated with major vascular injuries sustained during laparoscopic surgery between 2004 and 2020. Ten cases were identified, seven in New York City and three in Houston.

Of these cases, nine were female, and one was male. The ages ranged from 31 to 79 years (average, 56.6 years); five were Black, four were White, and one was Hispanic. The body mass index ranged from 26.7 to 46.7 kilograms per square meter (average, 37.3kg/m<sup>2</sup>). Nine cases underwent autopsy examination, and one underwent external examination only. The manner of death was accident in six cases and therapeutic complication in four cases (defined as when the death is caused by predictable complications of appropriate therapy and would not have occurred but for the medical intervention). The blood vessels injured during the procedure were the aorta (5), inferior vena cava (5), renal vein (4), superior mesenteric artery (1), hepatic vein (1), and left common iliac artery (1). In five cases, more than one major blood vessel was injured. The indications for surgery were cancer (4), cholelithiasis (2), uterine leiomyoma (1), and others (3)—including elective kidney donation. Five cases were pronounced dead on the operating table; and five cases survived for up to 12 hours.

Nine cases were obese or severely obese. Obesity was an initial contraindication to laparoscopic surgery in the 1980s, and although subsequent studies have shown it may be performed safely, it does present some technical difficulties, especially with the creation of the pneumoperitoneum.<sup>6</sup> Some modifications can be made, such as changing the initial trocar entry point. Another recognized risk factor for vascular injury is previous abdominal surgery with the presence of adhesions.

A meticulous dissection at autopsy, as for any penetrating injury of the body, can help delineate the injury pathway from the trocar entry point and identify the injured structures. This can provide helpful feedback to the operating surgeon and trocar designers and improve safety in laparoscopic procedures. The trocar device that caused the injury can be reported to the United States Food and Drug Administration (FDA) by the user to help track these fatalities.

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### Laparoscopic Surgery, Trocar, Vascular Injury



## H41 The Petechiae Enigma—Manual Strangulation in the Absence of Petechiae

J.C.U. Downs, MD\*, *forensX, LLC, Savannah, GA 31406*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the medical findings in manual strangulation, specifically including that petechial bleeds are neither diagnostic nor required for the diagnosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing that petechial bleeds are not diagnostic nor are they required for the diagnosis of manual strangulation.

Asphyxial deaths come in many forms at all stages of the respiratory process from the environmental content (environmental asphyxia) to the carriage of oxygen (choking) to the cardiorespiratory system for delivery (strangulation/hanging) to the cell for ultimate consumption (biochemical). Strangulation occurs by two major modes: manual and ligature. Both involve impingement on these processes, most always by means of vascular compromise.

Among the many findings which may be present in strangulation cases, damage to the hyoid bone and/or thyroid cartilage may be considered by the unfamiliar to be pathognomonic of manual strangulation and their presence required to make the diagnosis. Pathologists are well-versed in the meticulous dissection and observation required to document not only the presence or absence of such findings but also the many other findings which may be present, or absent, in manual strangulation. Petechial bleeds, especially those of the conjunctiva, are considered by many to be the *sine qua non* of strangulation—especially manual strangulation.

Common features referenced as “expected findings” in cases of manual strangulation include strap muscle bleeds, hyoid fractures, laryngeal fractures, facial petechiae, conjunctival petechiae, mucosal petechiae, and external neck trauma (inflicted contusions and/or abrasions and/or fingernail marks). In some forms of neck compression (i.e., the carotid sleeper hold also known as the Lateral Vascular Neck Restraint [LVNR]), the surface used is so broad that external and internal direct trauma is minimized as it is dissipated over larger areas. Since this force is applied lower and more broadly on the neck, observable damage to the bony and cartilaginous structures (including the airway) is not expected. Since this means of asphyxiation results in a rapid loss of consciousness, there is little time for struggle to result in secondary injuries. Properly applied, the LVNR would not be expected to produce many, if any, petechial bleeds.

While common in most strangulations, petechial bleeds may be inconspicuous or even absent in some types of strangulation and vascular constriction of the neck, such as the carotid sleeper hold. It takes very little pressure to occlude the venous return to the heart via the jugular veins and only slightly more to occlude the arterial supply via the carotid arteries. The net result is that, under the “right” circumstances, minimal-to-no directly observable damage may be expected at the site(s) of injury in such cases. If applied briefly, the LVNR can cause loss of consciousness and death within minutes or less. If released prior to death, the result of a properly applied LVNR may be strangulation with no documentable findings.

Through a series of cases of manual strangulation involving the carotid sleeper hold, this presentation will document the relative paucity of trauma that may be associated with such deaths.

The danger is that the novice or one speaking outside their area(s) of expertise may misinform the arbiter of fact by “eliminating” strangulation in subtle cases based on personal bias, misinformation, and misconceptions. In so doing, a practitioner may prevent truth in evidence by obfuscating relevant testimony. The net result being a miscarriage of justice.

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### Petechiae, Manual, Strangulation

## H42 Axonal Injury Is Detected by $\beta$ -Amyloid Precursor Protein ( $\beta$ APP) Immunohistochemistry in Near Instantaneous/Rapid Death From Head Injury Following Road Traffic Collision (RTC)

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**Learning Overview:** After attending this presentation, attendees will better understand that traumatic axonal injury can be detected in short survival if the brain looks unremarkable, the pathophysiology of axonal injury, and the limitation of diagnostic immunohistochemistry.

**Impact on the Forensic Science Community:** The axonal injury can be detected in the brain in those who died rapidly or near instantaneously following a fatal RTC. It is proposed that the early accumulation of  $\beta$ APP is probably due to the high magnitude of loaded forces in head injury resulting from RTC in this cohort, which could possibly cause higher proportions of primary axotomies and/or that the stretching axonal injuries are more severe causing quicker alteration in the axonal cytoskeleton. This presentation will impact the forensic science community by examining how this study's result is expected to have implications on the timing of head injuries in medicolegal practice, but caution is recommended to not overinterpret the results, which require close correlation with autopsy findings, circumstances of the incidence, and additional investigation.

**Introduction:** Timing of traumatic axonal injury is one of the challenging tasks in forensic neuropathology. It depends on assessing the morphological evaluation of axonal injury via demonstration of axonal injury by Hematoxylin-Eosin (H&E) stain, silver stain, and, more accurately, by immunostaining for  $\beta$ APP. The accumulation of  $\beta$ APP caused by axonal injury is an active energy-dependent process thought to require blood circulation and is therefore closely related to the patient's post-injury survival time. The earliest reported time that axonal injury can be detected by immunohistochemistry following traumatic brain injury by autopsy brain examination is currently 35 minutes.

**Goal:** To investigate if  $\beta$ APP staining for axonal injury can be detected in the autopsy brain of patients with near instantaneous/rapid death following RTC.

**Material and Methods:** This study involved retrospective examination of 49 deaths following RTC and one fatal single-occupancy aviation crash where there was reliable information concerning the time between the incident and the death. Thirty-seven patients (Group 1) died virtually instantaneously or very rapidly at the scene of the incident. A further three patients died after 30 minutes to 11 hours (Group 2) and eight patients died between 2 and 31 days after the RTC (Group 3). The brains from patients who died of instantaneous death due to sudden unexpected death in epilepsy (Group 4) were used as a non-traumatic control group. The brains were comprehensively examined, and a full set of tissue blocks from the cerebral cortex, white matter, corpus callosum, basal ganglia (including the internal capsule), cerebellum, and brainstem were sampled in all cases and immunohistochemically stained with  $\beta$ APP.

**Results:** The  $\beta$ APP immunoreactivity is demonstrated in minute amounts of variable pattern and low frequency in 35/37 brains of Group 1, in more intensity and frequency in 3/3 brains of Group 2, and 7/8 brains from Group 3, compared with no  $\beta$ APP immuno reactivities in all four brains from the control group (Group 4).

**Conclusion:** Axonal injury can be detected in the brain of those who died rapidly or near instantaneously following fatal RTC. The very early accumulation of  $\beta$ APP is possibly due to higher proportions of primary axotomies and/or that the stretch axonal injuries are more severe and cause quicker alteration in the axonal cytoskeleton.

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### Traumatic Axonal Injury, $\beta$ APP, Short Survival

## H43 Anaphylactic Death: A New Forensic Workflow for Diagnosis

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**Learning Overview:** The goal of this presentation is to suggest that through the use of the blood tryptase concentration together with the immunohistochemical investigation for anti-tryptase antibody in the lung, glottis, and skin samples (at the site of administration of the medication and contrast medium), it is possible to realize a diagnostic workflow as a gold standard of anaphylactic death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the necessity of proposing a diagnostic workflow as a gold standard in the diagnosis of an anaphylactic death.

**Background:** The term “anaphylaxis” was introduced in 1902 by Portier and Richet and referred to a serious, generalized, or systemic allergic or hypersensitivity reaction.<sup>1,2</sup> It can be a life-threatening or fatal clinical emergency with airway and circulatory impairments.<sup>3</sup> It is usually associated with skin and mucosal alteration (widespread hives, pruritus, swollen lips, tongue, uvula) and gastrointestinal disorders (vomiting, diarrhea, abdominal cramps).<sup>4</sup> In particular, anaphylaxis is due to a systemic reaction mediated by vasoactive amines released from mast cells and basophils sensitized by Immunoglobulin E (IgE). Conversely, anaphylactic shock is an anaphylactic reaction characterized by critical organ hypoperfusion after exposure to a previously encountered antigen.<sup>5</sup>

**Materials and Methods:** Eleven autopsy cases of anaphylactic death that occurred between 2005 and 2017 were investigated by the Departments of Forensic Pathology of the Universities of Foggia and Catania, Italy. Death scene investigation and autopsy reports, together with the information gathered from the police from the 11 cases of anaphylactic death were reviewed. Cases with weak or deficient information about the manner of death were excluded. Decomposed bodies were also excluded from the study. All procedures performed in the study were in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the relatives. Seven autopsies were carried out on males and four on females. Of the 11 cases, one had a history of asthma, one of food ingestion, two of oral administration of medications, six did not refer to any allergy history, and one subject was unknown. All the cases (100%) showed pulmonary congestion and edema; 7/11 (64%) of the cases had pharyngeal/laryngeal edema and mucus plugging in the airway; only one case (9%) had a skin reaction noted during the external examination. Serum tryptase concentration was measured in ten cases, mean value  $133.5\mu\text{g/l} \pm 177.9$ . Immunohistochemical staining for anti-tryptase antibody on samples of lungs, pharynx/larynx, and skin samples at medication injection sites revealed that all cases (100%) showed strong immunopositivity for anti-tryptase antibody on lung samples; three cases (30%) displayed strong immunopositivity for anti-tryptase antibodies on the pharynx/larynx samples; and in eight of the cases (80%), there was strong immunopositivity for anti-tryptase antibodies on the skin samples at the injection sites.

Currently, there is no specific forensic workflow in cases of death from anaphylactic shock. A systematic approach would allow forensic pathologists to arrive at a confident diagnosis of death from anaphylactic shock. In conclusion, a blood tryptase level ( $> 40\mu\text{g/l}$ ) together with strong positivity of anti-tryptase antibody immunohistochemical staining may represent reliable parameters in the determination of anaphylactic death with the accuracy needed for forensic purposes. The latter parameter may be particularly useful in the absence of a patient’s clinical or medical history. Thus, a diagnostic workflow as a gold standard in the diagnosis of anaphylactic death is proposed, with the evaluation of blood tryptase level in combination with immunohistochemical anti-tryptase staining.

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### Anaphylactic Death, Diagnostic Workflow, Toxicological Investigation

## H44 Neck Injuries Associated With Strangulation With Cable Ties

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the distribution of neck injuries in deaths due to asphyxia caused by strangulation with cable ties. Attendees will also gain insight into how the injuries are distributed in cases where the manner of death is considered to be suicide compared to cases considered to be homicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing how attendees consider the postmortem examination of cases succumbing to strangulation by cable ties. Attendees will be provided guidelines for how the autopsy of such deaths should be performed, focusing on the description of the arrangement of cable ties around the neck and the importance of neck dissection. The overall aim is to provide a scientific basis for the forensic pathologists' postmortem evaluations of strangulations with cable ties.

All medicolegal autopsies performed in Sweden between 2002 and 2019 in which the death was associated with strangulation with cable ties around the neck were examined. In Sweden, virtually all cases of suspected unnatural deaths are subject to a medicolegal autopsy by the governmental agency, the Swedish National Board of Forensic Medicine. Cases with more severe decomposition will be excluded. The aim of this presentation is to describe neck injuries and characterize those injuries in relation to the manner of death, that is, suspected homicide or suicide. The distribution of age, gender, toxicology results, and possibly type of psychiatric illness in the suicide cases will be presented and correlated to the distribution of injuries. A guideline for which findings should be noted during the autopsy, especially concerning the arrangement of the cable ties, will be presented.

This presentation will highlight an area of death investigation—strangulation by cable ties—in which the scientific basis for the assessment of the manner of death is sparse. In order to characterize injuries in the neck associated with strangulation with cable ties, large case series are needed. In the practice of forensic medicine, it is important that scientifically derived material exists that presents the distribution of neck injuries related to the manner of death and the arrangement of cable ties around the neck. Once the cable ties are applied, they are virtually impossible to remove by force of hand. Hence, there may be no other physical evidence on the body of a possible struggle, and the only physical evidence available to the forensic pathologist in the estimation of homicide or suicide is that provided by injuries in the neck and by the arrangement of the cable ties.

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### Injuries, Strangulation, Cable Ties



## H45 Otorrhagia in Strangulations: An Important But Often Underestimated Finding in Forensic Pathology

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge of strangulation cases in which otorrhagia is a useful sign for the diagnosis of death, representing a vital reaction of neck compression.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of auricle examination and otoscopy in mechanical asphyxia deaths (particularly in strangulation cases). As this case demonstrates, ear bleeding can be an important vital sign of neck compression and can aid in the diagnosis of strangulation, especially when a ligature mark is absent.

This case report is an unusual case of massive bilateral otorrhagia produced during a particular case of strangulation (death by garroting). In forensic practice, it is not always easy to understand if a person has died from strangulation as ligature marks are sometimes absent, especially when soft devices are used. The recommended physical examination must always include a thorough inspection of the head, neck, oral cavity, and eyes. In fact, facial congestion, petechiae of the skin or conjunctiva, scleral hemorrhages, and neck injuries are characteristic external signs. Hemorrhage from the ears is poorly described in mechanical asphyxiation and is an underestimated finding.

**Case Report:** An 87-year-old woman with senile dementia was found dead on her bed. Around the neck, the woman wore a handcrafted garrote consisting of a cloth belt and an apron, tied to the handle of a hammer. The external examination showed an ecchymotic mask and subconjunctival petechiae. The neck had a superficial cord imprint of about 2cm, but the ligature mark was not present. The front of the neck showed only small bruises, discontinuous abrasion, and petechiae. There were no lesions of the scalp or skull. External examination showed marked bilateral otorrhagia. The tympanic membranes were ruptured with bleeding into the external auditory meatus. Internal examination showed hemorrhage of the tongue base, a small hemorrhagic infiltration of the anterior neck muscles, and thyroid membrane petechiae, but no laryngeal osteocartilaginous injury. Dissection of the head and brain examination showed no fractures of the skull or petrous bone and no intracranial hemorrhages.

**Discussion and Conclusion:** Otorrhagia is generally associated with skull base fractures, lightning strikes, abuse in children, and diving accidents. In forensic medicine, ear bleeding is also described with drowning and is only exceptionally described in cervical compression. Furthermore, their formation mechanism is somewhat controversial.

A traumatic otorrhagia may represent a sign of vitality and helps in the diagnosis of death. The mechanisms that can explain otorrhagia in cases of neck compression are different. The middle ear is drained by two veins that flow into the internal and external jugular veins. The compression of the neck easily creates blockage of the jugular blood return, vascular congestion of the middle ear, and hemorrhage. The second mechanism is based on the respiratory effort against a closed glottis that can cause rupture of the tympanic membrane. Compression of the neck simulates closed glottis as the upper respiratory tract is tightly closed during strangulation. Therefore, laryngeal or pharyngeal obstruction causes an increase in pressure in the middle ear, through the Eustachian tube (which opens when the pressure of the pharynx increases). This increase in pressure could cause a middle ear injury and bleeding.<sup>1,2</sup>

This case represents one of the first reports of bilateral massive otorrhagia during a particular case of strangulation (i.e., death by garroting). The use of a soft medium (i.e., the apron and the cloth belt) did not result in an evident ligature mark. Otorrhagia was certainly a very useful sign and confirmed the death by mechanical asphyxia. Ear bleeding could represent an important sign of vitality and is related to the compression of the neck, in the absence of head trauma. In fact, this sign implies a respiratory movement and blood flow during the agonal period.

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### Otorrhagia, Strangulation, Garrote

## H46 The Reconstruction of the Dynamics in Slash Injuries: A Case of Attempted Murder

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**Learning Overview:** After attending this presentation, attendees will understand the role of the reconstruction of the dynamics in cases of attempted murder by slashing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how to evaluate the real intention of the killing by the aggressor in cases where the versions reported on the event are discordant or inconsistent through the study of the dynamics.

A sharp-edged weapon is characterized by a tip, with a penetrating ability, and a sharp edge. The mechanism of action is penetration, pressure, and/or severing, each of which cause very different injuries.<sup>1</sup> They are in fact divided into: tip, cut, and tip and cut.<sup>2</sup>

Slash injuries, on the other hand, are cuts produced by weapons which, in addition to being sharp, are characterized by a weight that produces a blunt injury. The coroner's task is to assess whether the injuries were inflicted by third parties. In cases of attempted murder, it is necessary to investigate the real intention of the aggressor to kill, not just to harm the victim, to confirm the crime of attempted murder. In these cases, it is often difficult to establish this intention as the versions of the victim and attacker could be discordant. Therefore, a precise reconstruction of the dynamics is essential. For this purpose, the literature considers essential the assessment of the crime scene, the location of the injuries, the number of injuries, the possible presence of defensive injuries, and the stages of the struggle.<sup>3</sup> This presentation describes an attempted murder with a slash in which the analysis of the injury to the victim allowed an accurate reconstruction of the dynamics.

A man was repeatedly hit by another man with a hatchet that caused widespread injuries. The victim tried to defend himself from the attacker. Soon after, the victim was transferred to the hospital. The attacker reported that he did not intend to kill the man, but that he only wanted to hurt him. The attacker attempted to prove this version by reporting that the victim had actually survived the struggle. The judge asked for an evaluation of the victim's injuries to establish the real dynamics of the event by comparing the victim's and aggressor's versions of the event. A medical examination was carried out on the victim. All lesions were analyzed, measured, and photographed. The topography of the lesions was reported on a special human model. The results of the visit were compared with the ultrasound and Computed Tomography (CT) scan results performed during the hospitalization of the victim. The visit revealed five lesions on different parts of the body. The victim was hit at the left region of the neck, at the left forearm, at the palm of the right hand between the second and third fingers, at the right forearm, and at the left thigh on the anterolateral surface. The analysis of the injuries made it possible to reconstruct the dynamics of the event and the phases of the attack. In fact, the injuries on the hands and forearms were attributable to attempts to defend himself. Therefore, it was possible to establish that the blows had been struck in a first phase on the left side of the victim, taking him by surprise. In a second phase, the blows were quickly inflicted in an anterior position with multiple attempts by the victim to defend himself in a situation of dominance by the aggressor. Only the use of the limbs provided a barrier that prevented the attacker from sinking fatal blows aimed at vital regions, such as the neck. The radiological examinations revealed that the injury to the neck was in fact very close to the nervous vascular structures. Therefore, the aggressor's intention to kill was clear.

The reported case shows that in cases of attempted murder, it is essential to analyze the topography of the lesions as soon as possible. The analysis of the event requires careful examination for defensive injuries on the limbs which, together with the radiological data, can constitute irrefutable evidence in court to clarify the stages of the struggle and demonstrate the real intention to kill.

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### Forensic Sciences, Slash Injuries, Murder

## H47 Physical Restraint and Neglect in the Elderly: A Forensic Case and Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will be able to describe the impact of forensic science in cases of restraint use in the elderly.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that restraint is not to be considered a therapeutic act and does not reduce the risk of falls in the elderly.

The elderly population is defined as people aged 65 years and over. The demographic trend of the past decades is characterized by an increase in the elderly population. In these subjects, restraint is justified by the high risk of falls and the general deterioration of health.<sup>1</sup>

This study was conducted through the review of the scientific literature and an analysis of studies relating to efficacy and risks related to restraint. Judicial inspection and autopsy were carried out in the death of an elderly woman; details of that death are reported here.

An elderly woman was found dead in her home. Scene analysis revealed that the body was completely burned by fire and that the fire involved only one room in the apartment. The relatives initially reported an accidental death by fire due to proximity of a shawl, worn by the woman, to a nearby stove. However, the stove was found on the balcony opposite the room where the fire had occurred. Furthermore, on the same balcony were found remains of a wooden chair, on whose arms burnt remains of wool laces were found. All the rooms in the apartment were examined. Fragments of string of a similar type and material to the remains found in the chair were found in garbage cans. At autopsy, the elderly woman's wrists demonstrated bruising from restraint. The analysis of all the data concluded the use of restraints of an elderly woman, who was closed in a room every day, without any surveillance by family members, tied to a chair located near a heating stove. The elderly woman lived with a caregiver who went away for many hours and left her tied to the arms of the chair. When the clothes caught fire, the woman could not escape and died from the combined effects of fire and inhalation of smoke (carbon monoxide).

Restraint means any action that limits an individual's freedom of movement and/or the normal use of the body, such that the victim cannot easily control or remove themselves.<sup>2</sup> The restraint can be: (1) physical restraint (reduction of the freedom through the use of a belt or other retention means); (2) a chemical restraint (administering drugs that reduce freedom of movement); and/or (3) environmental restraint (environmental modifications in which the victim lives in order to limit his/her movements).

The elderly population is vulnerable; therefore, they are often victims of restraint by family members or caregivers. In the elderly, restraint is justified by the general deterioration of health, space-time disorientation, increased risk of falls, and aggressiveness. Restraint is used due to the difficulty in managing the elderly person and the belief that it will reduce the risk of negative events. However, restraint in the elderly has many negative consequences: physical (related to immobilization such as ulcers, incontinence, muscle atrophy), psychological (depression, restlessness), and social.<sup>3</sup> Therefore, restraint should not be considered a therapeutic act in the elderly as it worsens health conditions and does not reduce the risk of falls. The elderly victims of physical restraint have greater cognitive impairment and worsening of global autonomy, with a reduction in the scores on the Barthel index. Furthermore, restraint violates the dignity of the person. The case reported here highlights the crucial role of the correct analysis of crime scene data even in apparent accidental deaths of the elderly. Restraint is related to high health risks for the elderly. The importance of family and social integration of the elderly in order to prevent the occurrence of accidents related to lack of control in elderly subjects and the need to limit the use of restraint to only those cases where it is indispensable for elderly safety have been demonstrated.

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### Forensic Sciences, Elderly, Restraint

## H48 Cranial Injury Pattern Analysis in the Reconstruction of a Homicide With a Hammer

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**Learning Overview:** The goal of this presentation is to show the fundamental importance of an adequate medicolegal expertise to elucidate the medicolegal etiology of death, namely in the investigation of a homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a very interesting case, initially cataloged by the police as a probable natural death, in which the medical-legal investigation came to demonstrate, unequivocally, that it was a homicide.

The death of a 76-year-old man, found in a remote rural area on a small access road to his home in an advanced state of putrefaction is reported. The body was found lying in a prone position, next to rural working tools and a wheelbarrow. The site was initially inspected by the police. Due to advanced putrefaction, with numerous larvae on the corpse, traumatic injuries were not identified. The remains were referred for a forensic autopsy with the information that it was a possible natural death, as the victim suffered from various cardiac pathologies.

Externally, the asymmetrical pattern of putrefaction (much more advanced at the head) suggested possible traumatic head injuries. At autopsy, seven blunt lesions on the scalp were identified. Examination of the skull revealed two depressed fractures that appeared related to each other, allowing the sequence of the impacts to be determined using Puppe's rule (observing a radiated fracture line that ends in another fracture line associated with another injury). Cranial fractures in the temporal and occipital regions were typical of fractures produced by a blunt instrument, like a hammer. It was clear that the death was a homicide involving skull fractures with associated cerebral hemorrhage, with no other relevant findings. Faced with this information, the police returned to the scene of the death and seized several tools as well as a hat that was next to the victim.

It was thus possible to identify the hammer used in the crime; it had blood stains and skin debris on its surface. There was a perfect match with fractures and striated bone lesions produced by the impact of the hammer handle on the skull. The seven injuries could be matched to the hammer and were likely produced by three impacts, demonstrating that one impact produced more than one injury. The correspondence of a tear in the hat, the blood stains, the scalp wounds, and the fractures observed allowed for the reconstruction of the mechanism of injury. The murderer (the victim's stepson) was later arrested and confessed to the crime (due to financial reasons).

This case study demonstrates the value of comparing injuries observed at autopsy, along with anthropological examination, to properly identify a murder weapon, the mechanism of injury, and thus conclude that a death was a homicide.

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### Homicide, Injury Pattern, Medicolegal Investigation

## H49 A Fatal Case of Retropharyngeal Abscess Following a Cervical Penetrating Wound

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**Learning Overview:** After attending this presentation, attendees will better understand the challenges constituted by potential infectious complications of cervical district injuries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a case of death due to retropharyngeal abscess following a penetrating wound at the neck.

A 56-year-old man with a clinical history of Parkinson's disease presented to the emergency room due to a penetrating wound at the anterior cervical region as a result of a reported domestic accident with a fire poker. Physical examination revealed a deeply penetrating wound that extended through the full thickness of the thyroid cartilage, reaching the laryngeal cavity. This finding was confirmed by laryngoscopy. Following disinfection and suturing of the wound, tetanus prophylaxis, antibiotics, corticosteroids, and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), the patient was discharged. After nine days, antibiotic therapy was extended due to purulent material in the larynx. The next day, the patient was found dead at home. A medicolegal autopsy was requested in order to determine the cause of death.

External examination revealed, at the level of the lower edge of thyroid cartilage, a linear scar 1.1cm in length, in a healing phase. The right laterocervical skin region demonstrated a greenish discoloration. At autopsy, a voluminous collection of purulent material of green-brown color was detected in the retropharyngeal region; the lesion was removed *en bloc* with the main structures of the neck, and subsequently fixed in 10% buffered formalin. Other organs were unremarkable for any significant findings.

Subsequent gross examination on the formalin-fixed organs allowed reconstruction of the tract previously produced by the foreign body. Furthermore, it was possible to better describe the walls of the abscess cavity identified during autopsy, which measured about eight centimeters in diameter, and carry out targeted histological sampling. Microscopic examination confirmed an inflammatory reaction while the wall of the abscess cavity consisted of connective tissue structurally subverted by widespread and intense necrosis, fibroblastic proliferation, and leukocyte infiltration. In the lungs, leukocyte infiltrates were found at the peri-bronchial level, suggestive of interstitial pneumonia; no other significant findings were seen in other organs.

This case report presents an atypical infectious complication of a penetrating wound to the neck. This complication is reported in pediatric subjects and only rarely observed and described, in the international scientific literature, in adults. The pathogenetic mechanisms involved in death can constitute a challenge for the medical examiner, especially in the absence of a full-blown septic picture. In the present case, the results suggest a mechanism of respiratory insufficiency based on a two factors: (1) by mechanical obstruction of the upper airways caused by the compression exerted by the abscess, and (2) by the reduction of respiratory exchanges secondary to infection in the lungs.

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### Retropharyngeal Abscess, Neck Injury, Autopsy Investigation

## H50 Around the World: A Short Case Series of Atypical Intracranial Bullet Tracks

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**Learning Overview:** While most bullet pathways within the brain follow a linear pathway, bullets may travel along the inner table of the skull and come to rest on the opposite side of the head, without directly causing deep brain injuries. The goal of this presentation is to show that penetrating intracranial gunshot wounds may travel along the inner table of the cranial vault and come to rest on the opposite side of the head without an exit wound and without penetrating the deep structures of the brain.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by recognizing a unique intracranial phenomenon. Bullet pathways in gunshot wounds of the head frequently are linear within the cranial vault, with a linear wound path between entrance site and the final resting place of the projectile. Although ricochets may occur, especially with smaller caliber projectiles or projectile fragments, these too tend to produce linear pathways between entrance and ricochet point, as well as ricochet point and the final resting place of the projectile. This presentation describes two cases in which projectiles entered the cranial vault and traveled along the inner table of the skull, coming to rest in the superficial brain on the side opposite the initial entrance site, with no deep brain injuries identified. This phenomenon may explain clinical situations wherein physical findings (entrance site) and radiographic projectile location suggest a pathway through the central brain, but where clinical findings do not support such a linear pathway.

**Proposition:** Penetrating intracranial gunshot wounds may travel along the inner table of the cranial vault and come to rest on the opposite side of the head without an exit wound and without penetrating the deep structures of the brain.

**Case Descriptions:** Case 1—A 19-year-old male sustained multiple lethal gunshot wounds, including of the head, following an altercation on a street corner. Paramedics noted a bleeding entrance wound in the left temporal region. Imaging revealed a bullet within the right posterior cranium. At autopsy, the medium-caliber (.38 special) projectile was noted to have entered the left temporal region and traveled along the inner table of the skull, stopping within the superficial right occipital lobe. There was no bullet track through the deeper aspects of the brain. Case 2—A 42-year-old male was found dead in an auto shop from a suicidal gunshot wound to his left submental chin. Imaging revealed a bullet within the right cranium. Autopsy revealed the large-caliber bullet traveled superiorly and entered the cranial vault via the left anterior cranial fossa. The bullet damaged the left frontal lobe, then traveled along the inner contour of the skull, crossing the midline before stopping within the right superficial parietal lobe. There was an absence of injury of the deep central structures of the brain.

**Conclusions:** The cases presented here may help explain potential circumstances in which a projectile is located radiographically on the opposite side of the head from the corresponding entrance wound, without evidence of deep brain injuries. The projectile may not have traversed the deep vital brain structures, and particularly in the absence of an exit wound, should not be assumed to have traveled linearly. Depending on the circumstances of the case, such an occurrence may help to explain the potential physical activity of a gunshot wound victim and/or the clinical/radiologic findings. The cases presented illustrate the possibility that bullets, even those larger than small-caliber bullets, may travel along the inner table of the skull before coming to rest on the opposite side of the head, without direct damage to deep brain structures.

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**Forensic, Intracranial, Firearm**

## H51 A Pen as an Intermediate Target Becomes a Secondary Projectile Causing a Unique Patterned Injury

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**Learning Overview:** The goal of this presentation is to help attendees better understand how intermediate targets, especially those within gun barrels, may become secondary projectiles and create unique patterns of injury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic pathologists recognize situations in which secondary projectiles from intermediate targets may have been involved in gunshot wounds. Recognition of these findings can support other autopsy findings and scene investigation.

**Introduction:** The phenomenon of intermediate targets becoming secondary projectiles in gunshot wounds have been well described in the literature.<sup>1-3</sup> However, cases in which intermediate targets within gun barrels—that are not bullets—are a rarity. In general, when a bullet strikes a non-resistant intermediate target, assuming the main mass of the bullet is intact, it produces a single entrance wound with fragments of the intermediary target scattered around or within the entry wound.<sup>1</sup>

**Case:** The case involves a 16-year-old male and his father, who was manipulating a .40 semiautomatic handgun to demonstrate how the weapon worked. At one point, the father had placed an ink pen in the barrel of the firearm and pulled the trigger, ejecting the pen out the barrel. His son saw this and wanted his father to launch the pen once again. The father placed the pen in the barrel, pulled the slide back and did not notice that a live round entered the firing chamber. The weapon was discharged causing fatal injury to his son. Autopsy findings revealed a single entrance wound to the left chest where a fully jacketed, mildly deformed projectile was retrieved from the decedent's back. Over the left abdomen, multiple scattered superficial abrasions were present along with a 7 x 1.5cm Y-shaped patterned abrasion with central clearing of the lower portion of the wound was found. Surrounding the abrasions were thick, viscous splotches of black pigment consistent with pen ink that was also found on the bilateral hands, forearms, and on the front panel of the decedent's t-shirt. Upon close examination of the Y-shaped abrasion, the central clearing appeared to outline internal components found in pens, such as the spring and ink chamber.

**Discussion:** In this case, a pen, the intermediate target, became a secondary projectile, striking the decedent and creating a patterned abrasion. A similar situation has been described in a case report by Ellis who found a metal barrel-cleaning brush and bullet in a single-wound suicide.<sup>4</sup> In that case, the brush, which was inside the firearm barrel, created the initial wound with the bullet in tandem entering the same wound. This study's case represents a similar mechanism of action; however, the intermediate target was likely partly destroyed and fragmented as it was pushed out of the barrel by the bullet, as evidenced by the scattered abrasions and ink splotches around the large Y-shaped abrasion. Identification of the Y-shaped abrasion as the pen corroborated with the rest of the autopsy findings and scene investigation.

**Conclusion:** This case highlights the importance of understanding how intermediate targets within a gun barrel can become secondary projectiles when propelled by the primary projectile. Recognition of unique patterns of injury in gunshot wound cases can help forensic pathologists in identifying this phenomenon.

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### Secondary Projectile, Intermediate Target, Patterned Injury



## H52 The Alteration of the Appearance of a Gunshot Wound by the Use of a Hemostatic Agent

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**Learning Overview:** After attending this presentation, attendees will have learned about another artifact that can potentially cause misinterpretation of gunshot wounds

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reinforcing the necessity of receiving pertinent information found or occurring at a death scene for correct interpretation of autopsy findings

A person was found with an apparent self-inflicted gunshot wound of the head. Emergency medical services were called, and law enforcement arrived first on the scene. The victim was transported to a local hospital, but death was pronounced shortly after arrival. Initial investigation found that no medical intervention had occurred, including resuscitative attempts. Additionally, no medical treatment had happened at the scene of the event per the responding law enforcement official. It was reported that a 9mm handgun was used, since this type of gun was found next to the body. When the body was examined in the morgue, the head had been wrapped with gauze, which had been done by law enforcement at the scene. After removing the gauze, a contact gunshot wound with soot, radiating lacerations, and a muzzle imprint on the right temple was noted; however, several small, tan, uniformly round, pellet-like objects were at the edges of the wound, within the muzzle imprint of the wound, and within the wound track. Available scene information provided no answer as to the nature of these pellet-like objects. Concern was raised that ammunition was used, possibly “snake shot,” that was not consistent with use of a 9mm handgun. Postmortem radiologic exams were performed, which increased confusion since the pellet-like objects were not radiodense and, therefore, not metallic. After time-consuming investigation and interviews with the responding law enforcement official, it was discovered that the officer had used an agent called QuikClot® on the wound and had wrapped the head with gauze prior to transport of the body to the hospital.

QuikClot® is a hemostatic agent used by military, law enforcement, and other first responders when encountering wounds that are freely bleeding. According to this product’s website, this agent consists of small pellets “impregnated with kaolin that accelerates the body’s natural clotting ability and produces no exothermic reaction.”<sup>1</sup> Other products that are used to promote hemostasis out in the field include material designed as a folded dressing embedded with expanding sponges, material consisting of high-surface-area flakes, and material made from chitosan that adheres to red blood cells to promote platelet activation. Use of hemostatic agents is well known to trauma surgeons within a hospital or military setting, but its use is not often encountered at death scenes in civilian settings. This presentation will discuss what QuikClot® is, what it looks like within a wound, and how its unexpected presence may cause confusion when evaluating gunshot wounds.

### Reference(s):

1. QuikClot®. Power to Stop Bleeding®. <https://quikclot.com/>.

### Gunshot Wounds, Hemostatic Agents, Death Investigation



## H53 Embolization of Radically Invasive Projectiles (R.I.P.<sup>®</sup>)

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**Learning Overview:** The goal of this presentation is to illustrate an atypical complication that can result from injury from a G2 R.I.P.<sup>®</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing an atypical presentation of injuries from unusual ammunition, thus bringing awareness to an atypical and rare complication.

A 34-year-old man was brought to the emergency room after receiving a gunshot wound to the right side of the chest. An X-ray of the chest revealed atelectasis and metal foreign bodies. He was persistently hypotensive and was taken to the operating room for exploratory laparotomy. Findings included a liver laceration and diaphragmatic rupture, which were managed with liver packing and diaphragmatic repair. The patient was subsequently noted to have ST-T changes on 12-lead Electrocardiogram (EKG) with elevated cardiac troponins, consistent with myocardial infarction. Echocardiogram revealed normal left and right ventricular function. His cardiac complications were managed medically with aspirin and atorvastatin as he was not a candidate for cardiac catheterization. The hospital course was complicated by bradycardia, hypotension, and hypoxia and despite management, the patient died ten days post-admission. No projectiles were recovered during the hospital stay. The decedent was sent to the Office of the Chief Medical Examiner for an autopsy.

On full-body imaging, one bullet and eight trocars were identified throughout the right side of the thorax and abdomen. Of the eight identified, one trocar was seen in the cardiac silhouette. At autopsy, seven trocars were located in the liver and right lung. The injuries included right lobe of the liver and lower lobe of the right lung lacerations. On dissection of the right coronary artery, one trocar was lodged in the vessel proximally, with surrounding thrombus formation resulting in complete occlusion of the vessel lumen. The proposed path for the trocar is that it likely injured the right lung and gained access to the left side of cardiac circulation through the pulmonary veins, then traveled through the left atrium, left ventricle, aorta, and finally, the right coronary artery ostium. The trocar traveled approximately one centimeter until it was limited by the diameter of the coronary artery. All eight trocars were retrieved. Though there was extensive organ injury caused by the trocars, the proposed terminal event resulted from the single trocar lodged in the right coronary artery, subsequently becoming a nidus for thrombosis of the vessel. Corresponding clinical findings at the final stages of medical care further support the mechanism.

R.I.P.<sup>®</sup> rounds are becoming increasingly popular, and thus presentations of injuries from this ammunition are becoming more common. This type of ammunition proves itself to be challenging to manage clinically and at autopsy. A typical presentation of injuries from such ammunition would result in multiple internal injuries with complications of blood loss; however, presentation of embolization with coronary artery occlusion and resulting myocardial injury is rare.

Bullet and shotgun pellet embolization have been reported. With the introduction and availability of this invasive ammunition, awareness of the eight extremely sharp, small trocar fragments is of utmost importance to forensic pathologists, clinicians, and surgeons. The small size of the trocars allow for not only extensive collateral tissue damage, but also delayed injuries from embolization and further travel throughout the body. Myocardial infarction in patients injured by R.I.P.<sup>®</sup> rounds should prompt full evaluation of the coronary artery system, even if major injuries and bleeding have been treated. Therapy in this setting would include conservative management such as aspirin and beta blockers and more aggressive management in removal of the trocar. Conservative management was unsuccessful in this case.

Delayed deaths following injuries by R.I.P.<sup>®</sup> projectiles should raise suspicion for the possibility of trocar embolization and its consequences. Familiarity with this unique ammunition and its various patterns of injury and complications is essential when presented with one of these challenging cases, not only in the forensic community but also in the surgical and clinical communities.

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### Embolization, Ammunition, R.I.P.<sup>®</sup>

## H54 Gunshot Defense Wounds: The Need for a Classification

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**Learning Overview:** After attending this presentation, attendees will understand how gunshot murders can be associated with the presence of injuries located on forearms and hands attributable to defense attitudes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stressing the importance of the categorization of gunshot defense wounds as well as in cases of stab defense wounds.

Defense wounds are injuries caused by the victims attempting to defend themselves. In blunt or sharp force assaults, defense injuries are typically seen on the arms and hands; rarely, they can be found on the feet or legs. Defense wounds in firearm injuries are rarely reported in the literature and occur when an arm is raised in a desperate attempt to shield the trunk and head from the blast.

**Case 1:** A 33-year-old male was found dead with multiple gunshot wounds. The circumstantial information revealed the shooting followed an altercation between the victim and one or more perpetrators. A complete medicolegal autopsy was performed. Wound locations, appearances, paths of the missile, injuries produced, and exit wounds were reported. The gunshot wounds are summarized thus: (1) left upper chest (entrance wound) → left lung → heart → diaphragm → aorta (retained bullet); (2) right abdomen (entrance wound) → left sacrum (retained bullet); (3) back of right hand (entrance wound) → palm (exit); and (4) channel wound on left hand → lower forearm (entrance wound) → upper forearm (exit). It was argued that the second and third trajectories could be due to a single gunshot.

**Case 2:** A 50-year-old man was killed by his son with multiple sawed-off shotguns. The wounds were thus analyzed: (1) left scapula (entrance wound) → upper lung lobe left → upper tract of the descending thoracic aorta → fracture of the second and third ribs → left anterior thoracic region (exit); (2) large gaping wound on lower left forearm (entrance wound) → upper forearm (exit); and (3) channel wound on lower left forearm (entrance wound) → upper forearm (exit).

In assaults of any kind, the natural reaction of the victims is to protect themselves. Limbs used for protection can themselves be injured. These defense wounds may be of considerable medicolegal significance as they indicate that the victim was conscious, at least partly mobile, and not taken completely by surprise. The classic position of defense wounds is on the forearms and hands, which can be instinctively raised to protect the eyes, face, and head. Defense wounds from knives consist of cutting wounds, as the blade is drawn across the tightly applied skin. Defense wounds also occur in firearms injuries, where an arm is raised in a desperate attempt to shield the trunk or head from the blast. The bullets may not stop on the impacted surface but may instead penetrate the tissues. The exact self-defense movements are extremely difficult to reconstruct as the victim was conscious and capable of many movements; his/her upper limbs could perform movements of adduction, abduction, extension, flexion, circumduction, rotation, supination, or forward and backward movement. The fundamental concepts of firearm pathology using characteristics of entry wounds (round shape, diameter smaller than bullet diameter) and exit wounds should still be applied. In murder cases, an accurate reconstruction of the events should include classifying gunshot defense wounds. These cases highlight the need for classification for gunshot defense injuries into passive or active categories. Passive gunshot defense injuries could be used to describe when the victim raises their hands or arms for protection, while active defense wounds would be when the victim tries to seize the weapon or the attacker's weapon-holding hand.

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### Forensic Ballistic, Gunshot Murder Cases, Gunshot Passive Defense Injury



## H55 Suicide in Older Adults: A Retrospective Study in Cook County, Illinois

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**Learning Overview:** After attending this presentation, attendees will understand the incidence and circumstances surrounding suicides in older adults (65 years of age and over) that were referred during the past five years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of suicide deaths in the elderly in an urban medical examiner's population, which may help determine risk factors, victim demographics, methods, and scenarios to help death investigators deal with this complex issue.

Suicide in older adults is a principal public health issue in many countries. The risk of suicide increases with age in almost all countries, and suicide rates reach 48.7/100,000 for older Caucasian men in the United States. Older adults may be more prone to self-harm behavior because of the higher prevalence and coexistence of financial issues, social isolation, bereavement, dependence, physical and mental illnesses, etc. In addition to this, when they decide to commit suicide, older adults make more lethal suicide attempts than younger subjects.

A retrospective study was conducted on suicides in subjects over 65 years of age based on the police investigation, autopsy, and toxicology reports collected from the Cook County Medical Examiner's office between January 1, 2015, and December 31, 2019. All of the cases were analyzed as to age, race, sex, suicide method, year of the suicide, toxicology results, medical/psychiatric history, social history, and presence of a suicide note at the scene.

The suicide cases in victims 65 years and older totaled 354, accounting for 15.2% of all suicides reviewed and for 4.4% of all the deaths in subjects over 65 years old in the same five-year period. Of these 354 cases, 84 (24%) were fully autopsied, 50 (14%) underwent head-only autopsy, and 220 (62%) were externally examined. The ages of suicide victims ranged from 65 to 95 years, with a mean age of 75. Males were 292 (82.5%) of the victims and females 62 (17.5%). The vast majority (300 cases) were Caucasian, 30 were African American, 22 were Asian, and 2 were "other". The male-to-female and Caucasian-to-African American ratios were 4.7:1 and 10:1, respectively. In 2015, 71 deaths occurred; in 2016, 58; in 2017, 72; and in 2018, 66. In 2019, the highest number of suicides (87) was registered.

Gunshot wound was the most common method of suicide, accounting for 51.4% of the cases. Other methods included hanging (17.8%), overdose (11%), fall from height (6%), plastic bag suffocation (4.8%), stab and incised wounds (2.8%), carbon monoxide poisoning (2.5%), train striking pedestrian (1.7%), drowning (1.7%), and disruption of dialysis catheter (0.3%).

This present study shows that the most common elderly suicide victim is a Caucasian male with an average age of 75 years, and the most common method is a gunshot wound. This study's results, including circumstances surrounding the deaths, toxicological data, and medical/psychiatric history, will be presented to the attendees.

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### Suicide, Elderly, Autopsy



## H56 How (Not) to Pop a Cyst at Home: Heart and Lung Perforation After Needle Insertion Into the Chest Wall

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**Learning Overview:** The goal of this presentation is to bring awareness of careful scene examination in similar cases and to underscore the importance of postmortem imaging.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness of the unusual circumstances that warrant careful examination of the scene, alerting the autopsy personnel to be cautious.

Self-inflicted foreign bodies in the heart and chest cavity are rarely encountered in medical practice. There have been mainly a few case reports of cardiac injuries inflicted by sewing needles or similar objects, either self-inflicted or accidental in the literature. The majority of the reported cases involve psychiatric history in the affected individuals. The reported accidental cases of needles finding their way to the heart are mainly iatrogenic, occurring, for example, after a needle decompression of a suspected traumatic pneumothorax in the pre-hospital setting. The incidence of similar cases in the forensic setting is unknown. A case of a young man who was attempting to self-treat a cyst with a piercing needle at home and lost control of the needle is presented.

The decedent was a 31-year-old male with a history of developmental and bipolar disorder who lived with his parents. On the day of the incident, his mother awoke to him screaming that he was trying to “pop” a cyst on his chest, but the “needle went all the way in.” He was bleeding from the chest and collapsed to the floor. On his computer, there was an instructional video for “popping” cysts. An empty wrapper from a piercing needle was discovered on the floor of his bedroom. Examination of the body at the scene revealed a bleeding skin defect in the sternal area, but the needle was not observed.

Autopsy showed a large abrasion on the right side of the chest with a central 1/16" puncture site and multiple clustered superficial punctures on the lateral and medial aspects. Per office protocol, a Computed Tomography (CT) scan was performed that showed right hemothorax, hemopericardium, and right pneumothorax and a 2" long needle that was completely embedded in the right third intercostal space. The tip of the needle projected at the level of the right atrial auricle, passing through the middle lobe of the right lung.

The cause of death was perforating injuries of the right lung and heart, and the manner of death was classified as accident.

While there were clear indicators in this case that a piercing needle was used, the needle was not visible externally. Careful examination of the scene, postmortem imaging, especially CT, was invaluable in revealing the exact location of the potential sharp object before it was dislodged and also alerted the autopsy personnel to be cautious.

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### Hemopericardium, Hemothorax, Self-Inflicted

## H57 Self-Immolation in a Dumpster

Wendy M. Gunther, MD\*, Office of Chief Medical Examiner, Tidewater District, Norfolk, VA 23510-1046; Gary Zientek, MD, State of Virginia, Norfolk, VA 23510

**Learning Overview:** After attending this presentation, attendees will recognize the characteristics of suicide by self-immolation, review burn intensity and distribution from accelerant use, and evaluate the significance of a slight elevation of carboxyhemoglobin in self-immolation deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by documenting a death by self-immolation in a dumpster and reviewing characteristic autopsy and toxicology findings when investigation and autopsy support a manner of suicide, rather than homicide followed by attempted concealment.

Bodies found on fire in dumpsters may be homicide victims with postmortem attempts at concealment. This case report details the scene investigation and autopsy of a suicidal death by self-immolation in a dumpster.

Firefighters called to a fire burning in a dumpster behind a business discovered a female body on fire in the dumpster, with very little other trash, most of it charred; she was pronounced dead on scene. She carried no identification; her clothing was lost to charring; a large tattoo on her left arm remained intact. She was recognized as similar in appearance to a woman who had been seen sitting crying on the curb in front of the store, about 20 minutes earlier.

Store surveillance video showed her sitting on display furniture, then stealing two bottles of charcoal lighter fluid from another display, followed by walking toward the area where the dumpster was located. Within minutes, smoke was seen rising from the area. No other person entered the area before the firefighters arrived.

At autopsy, she had deep burns to the lower extremities and back; there was charring of the left buttock and across the posterior legs. Superficial burns of the anterior torso and upper extremities were observed; the head was mostly spared. Soot deposits were noted across both the front and the back of the body. She had no detectable injuries to her wrists, shoulders, mouth, or face. Internal examination showed a small amount of mucoid soot in the trachea; there was no trauma or significant natural disease. Identification by fingerprint comparison gave her age as 41 years. Postmortem toxicology demonstrated no drugs of abuse or ethanol. The carboxyhemoglobin was modestly elevated at 14% saturation. Death was attributed to thermal injuries, and the manner was deemed suicide. No suicide note was discovered.

Self-immolation is an uncommon suicide method in Europe and the United States.<sup>1,2</sup> Decedents in Greece who committed suicide by self-immolation were predominantly elderly, often showed poorly controlled mental disorders (mostly affective and adjustment disorders), and usually chose outdoor locations that were either secluded or private.<sup>1</sup> This decedent chose to perform her self-immolation in an outdoor location that was secluded behind a building. A paper aggregating 46 self-immolation suicide reports found that European and North American victims were most often in their 30s and 40s; 10 out of 18 victims showed carboxyhemoglobin below 10% saturation, while several of the remaining eight were at low concentrations from 10.5% saturation upward, attributed to flash fire deaths.<sup>2</sup> Self-immolation may be performed with the aid of accelerants; Spitz reported that this often results in patchy charring, with some areas charred deeply and other areas significantly less so; even low levels of carboxyhemoglobin are significant in flash fire deaths.<sup>3</sup> A low carboxyhemoglobin level implies rapid death from airway damage and extreme heat flux; carbon monoxide accumulation from open flames may also be impaired by excellent ventilation.

The autopsy findings reported here, supported by the 14% carboxyhemoglobin saturation, are consistent with death from thermal injuries sustained in a flash fire; the distribution of the burns is consistent with self-application of accelerants. Although no laboratory analysis was performed for accelerants, the stolen containers, the patchy charring and burn distribution, and the low carboxyhemoglobin are supportive of accelerant use in a self-immolation suicide. The video evidence provided final confirmation that this suicide by self-immolation in a dumpster was not a homicide followed by attempt at concealment of the body.

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2. Simonit F., Da Broi U., Desinan L. The role of self-immolation in complex suicides: A neglected topic in current literature. *Forensic Sci Int* Jan 2020; 306(11073). <https://doi.org/10.1016/j.forsciint.2019.110073>.
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### Forensic Sciences, Self-Immolation Suicide, Dumpster Fire

## H58 Pre-Pandemic Trends in Suicide: New Mexico, 2009–2018

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**Learning Overview:** The goal of this study is to examine trends in suicides in the state of New Mexico over the ten-year period that preceded the coronavirus pandemic.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring demographics of suicide cases in New Mexico as well as establishing known trends of suicides in the pre-COVID-19 pandemic era for future comparison with suicide data collection after the arrival of COVID-19 in the United States.

Nationwide, suicide is the tenth-leading cause of death and the second-leading cause of death in 10- to 34-year-olds.<sup>1,2</sup> Suicide rates have been increasing in the United States over the past decade, and, in 2018, the suicide rate was 14.8 per 100,000 population, the highest since 1938.<sup>1</sup> According to the American Association of Suicidology, suicide is a major cause of morbidity and mortality for New Mexicans, with the state's 2018 crude suicide rate (25.6 per 100,000 population) being significantly higher than the national rate as well as the highest of all 50 states and the District of Columbia.<sup>1</sup> To assess suicide trends in the state of New Mexico, the Office of the Medical Investigator (OMI), the state's centralized medical examiner agency, conducted a retrospective analysis of all deaths with a manner of suicide from January 1, 2009, to December 31, 2018. Categorical variables were analyzed using either chi square or Fisher exact tests, and continuous variables were analyzed using *t*-tests and Analysis of Variance (ANOVA), as appropriate. Results were considered statistically significant if the *p*-value was 0.05 or less.

A total of 4,467 deaths were identified by the OMI's electronic database with suicide as the manner of death between 2009 and 2018. Analysis of these deaths revealed a 34% increase in suicides over that time period from 18.8 per 100,000 in 2009 to 25.2 per 100,000 residents in 2018, a statistically significant increase ( $p=0.001$ ). Of these deaths, 2,934 received a full or partial autopsy and 1,510 received an external examination. Analysis of gender was similar to national trends with males comprising 76% of the total suicidal deaths and women comprising 24%, with no significant difference in mean age by gender. Examination of race/ethnicity over the study period revealed significant differences in both racial/ethnic distribution and mean age by race ( $p<0.001$  and  $p<0.0001$ , respectively). Overall, White Non-Hispanic individuals encompassed the majority of suicidal deaths (64.6%) with a mean age of 50.6 years, followed by White Hispanic individuals (26%) with a mean age of 39.6 years and American Indians (7.1%) with a mean age of 32.8 years. From 2017 to 2018, suicides among American Indians increased by 45% (2017,  $n=33$ ; 2018,  $n=48$ ), and pediatric suicides (age range: 9–18 years), which had an unusual peak in 2017, decreased by 27% (2017,  $n=41$ ; 2018,  $n=30$ ). High school-aged children were at highest risk of suicidal death in the pediatric age groups consisting of 76% of the total pediatric suicidal deaths, followed by middle school-aged children (18%), and grade school-aged children (6%). There was a statistically significant increase ( $p=0.00001$ ) in suicide deaths of decedents aged 60 and older from 2009 ( $n=80$ ) to 2018 ( $n=150$ ), with a 12% increase from 2017 ( $n=134$ ) to 2018 ( $n=150$ ). The most common methods of suicide included use of firearms (52.7%), hanging (22.6%), and ingestion or injection of illicit or prescription drugs (15%).

This examination of suicidal deaths highlights observed trends and vulnerable groups within New Mexico's populace prior to the sweeping changes in societal norms resulting from COVID-19. As the pandemic is likely to increase risk factors for suicide, including financial stressors and social isolation, a focus of preventative resources on these vulnerable groups, especially adolescents and the elderly, is necessary to prevent further exacerbation of these trends.

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1. Facts & Statistics. *American Association of Suicidology*. Accessed September 20, 2020.
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### Suicide, Epidemiology, Method of Suicide

## H59 The Chainsaw Massacre at the Beginning: An Unusual Suicide With a Jigsaw at the End

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**Learning Overview:** The goal of this presentation is to underline the importance of cooperation between the forensic pathologist, the policemen, and the engineering analysis; this is fundamental in order to collect all the evidence necessary to solve complex and atypical cases, such as the presented case. Without evidence, no truth is possible to be reconstructed, so no reliable judgement can be reached.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that, despite the autopsy being an essential tool to provide scientific and forensic evidence, a correct interpretation of injuries, and of the mode of production (murder, suicide, accidental), the role of the forensic pathologist from the crime scene investigation is fundamental. The crime scene investigation carried out by a critical forensic mind and by an expert team is an essential instrument in the research and collection of evidence and samples.

According to the World Health Organization (WHO) fact sheet of September 2, 2019 on suicidal rates, it has been estimated that about 800,000 people die from suicide each year, constituting the third-leading cause of death between 15–19 years.<sup>1</sup> The most frequent method of completed suicide in the general population is due to pesticide self-poisoning, accounting for around 20% of suicides, more typical of the rural areas. After this, hanging and gunshot injuries are the most common suicide methods.

Suicides completed through the use of saws seem to be exceptionally rare and are considered atypical events; indeed, they appear to be most frequently accidental and homicidal events. Among saws, electric ones are even rarer.

The presented case concerns a suicide completed with a jigsaw, a particular type of electric saw, and seems to be the only one reported in the scientific literature. A 36-year-old obese woman was found dead in her house near Rome, Italy. Police officers and forensic pathology were immediately alerted to investigate the crime scene. The young woman was found lifeless, lying on the sofa in the living room. A series of blood drops were also found on the floor through the path to the bathroom. A bloody orbital jigsaw was plugged in and was in the off mode on the sink. Police officers found out that the woman had bought the jigsaw just the day before she was found dead. Her medical history revealed she was affected by depressive disorder. Prior to the autopsy, postmortem radiological examination was carried out. On the anterior side of the neck, there was a large lacerated wound, which involved the lateral surfaces of the neck with a total length of about 28cm and a maximum width of about 5cm. This wound was widely separated, irregular, excoriated, bruised, and had blackish margins. Neck dissection (according to the Adam technique, modified by Pomara-Fineschi) revealed a lack of platysma muscle; injury of the superficial muscle bundles of the sternocleidomastoid muscles bilaterally, in the context of hemorrhagic infiltration; and hemorrhagic infiltration at the level of the origin of the omohyoid, sternohyoid, and sternothyroid-thyrohyoid muscles, bilaterally. Another focal area of hemorrhagic infiltration affected the right lobe of the thyroid. The thyroid cartilage had a large, full-thickness, transverse laceration. When opening the larynx and trachea, abundant blood was observed. The cause of death was attributed to hemorrhagic shock with blood aspiration. The alcohol blood test was positive and the toxicological test was negative for other common abused substances. The integration of all the information concluded the death was a suicide.

Due to the rarity of this injury, a review of the literature is also provided to compare the salient features between the scientific data and this case.

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### Jigsaw, Suicide, Multidisciplinary Approach

## H60 A Rare Suicide by a Homemade Explosive With Multiple Suicide Notes: A Case Report and Systematic Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will understand how the construction of an illegal homemade explosive device may be very dangerous, especially in psychiatric patients with a high risk of suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the need for monitoring the production of homemade weapons by patients, through the collection of letters or diaries, in order to recognize promptly suicidal ideations and to prevent fatal events by these devices.

In recent years, the production of homemade weapons for recreational purposes has become increasingly widespread. Currently, the construction of these illegal devices is very simple thanks to the advent of numerous websites that explain how to produce a weapon with ease. Unfortunately, the risk associated with this phenomenon is poorly known. Several cases of suicide with home-manufactured firearms have been described in the literature while suicides with homemade explosives remain scarcely reported. A rare suicide of a patient with delusional depression is reported. The forensic investigations showed that the man had triggered a homemade explosive device, leaving dozens of suicide notes in which he announced his suicide. The case, presented in the context of a systematic review of the literature on suicides by explosives, demonstrates the need for monitoring the production of homemade weapons in psychiatric patients at high suicide risk. Monitoring of letters and transcripts could prove useful to reduce the risk of suicide in patients who have easy access to these means.

An elderly man was found dead outside his home. Neighbors said they heard a loud roar and contacted emergency services on suspicion of a gas leak. Family members reported that the man was passionate about building explosives and that he would soon have to undertake radiotherapy.

A crime scene investigation was carried out at the victim's home with photographic surveys. All the letters as well as the explosives present at the scene were analyzed. An autopsy was then performed. All organs were photographed, examined, and fixed in 10% formaldehyde. Biological fluids were collected for toxicological investigations. The data were compared with a systematic review of the literature about suicides by explosives. The PubMed® National Center for Biotechnology Information (NCBI) search engine was used by analyzing all the cases published in the English language between 1990 and 2020 through the key words: suicide AND (bomb OR explosion OR explosive). Papers concerning terrorist suicides or chemical explosions were excluded.

The crime scene investigation revealed a quantity of homemade pyrotechnic material equal to 500 grams in the man's bedroom. The analysis of the same room also showed about 20 suicide notes, a large part of which were hidden under the pillow of the bed; the others were kept in the trouser pockets worn by the man at the time of death. Autopsy showed traumatic destruction of the chest and abdomen with heart rupture and hemothorax. The examination of the abdomen revealed a clear line of hemorrhagic infiltration between the upper and lower abdominal quadrants attributable to the effect of the explosion, with hemoperitoneum and liver destruction. The toxicological investigations showed positivity for phenobarbital, whose blood concentration was equal to 17mcg/mL, as per drug therapy.

In solving the case, suicide notes were fundamental because they revealed a state of severe depression with obsessive and delusional suicidal ideation, which had been going on for several weeks. In accordance with the testimonial information, the man spoke of a cancer with a poor prognosis for which he should have undergone radiotherapy a few days later. The collected data were compared with the results of the literature review. Twenty-one published suicide cases were found from 1990 to 2020, with all male victims, aged 20–76 years (mean age of 37.6). In only three papers was the use of homemade explosives described.<sup>1-3</sup> Compared to the literature, the reported case is certainly unusual, both in relation to the home-manufactured weapon used and to the damage it produced. In particular, the autopsy evidence suggested that the victim had triggered the device by holding it in contact with his chest, using the right hand. The manner of the event was also confirmed by the numerous suicide notes found in the trousers and in the house, which showed suicidal thoughts that had been going on for weeks. This work demonstrates how the construction of illegal homemade weapons could be very dangerous, especially in people suffering from psychiatric disorders. For this reason, greater surveillance is emphasized, through the collection of letters and diaries, as tools to recognize suicidal ideations and to prevent fatal events by these devices.

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### Forensic Sciences, Homemade Explosive, Suicide Note



## H61 Multiple Suicidal Gunshot Wounds in Italy: Case Reports and Medicolegal Considerations

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**Learning Overview:** After attending this presentation, attendees will have improved their knowledge about the medicolegal investigations in cases of multiple gunshot wounds in order to establish the mechanism and cause of death and, therefore, to differentiate the suicidal, homicidal or accidental manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing two case reports of uncommon multiple Self-inflicted Gunshot Wounds (SGW). In this context, the important role of the death scene analysis, postmortem Computed Tomography (CT), and autopsy findings and clinical-circumstantial data were emphasized. Additionally, a presentation of 3D reconstruction of the death scenario is provided to better clarify the shooting dynamics.

**Case 1:** An 81-year-old male was found dead sitting in a chair on the veranda of his own house by his relatives (his wife and her cousin). During the death-scene investigation, blood was found on clothes, on the chair, and on the floor under it. Two round lesions were observed on the anterior surface of the t-shirt and below the left hemithorax, and one round lesion on the posterior surface of the chest, the above corresponding t-shirt area, and the back of the chair. The right hand showed some characteristic blood spatter. A semi-automatic pistol (Beretta® model 70, .32 Automatic Colt® Pistol [ACP]) containing five cartridges, with a cocked hammer, was found on the floor between the left foot of the subject and the left anterior leg of the chair. Two cartridge cases were found on the floor, behind and in close proximity to the body. No mental illness or relevant organic pathologies were identified. Social and family histories did not reveal any salient information. Postmortem examination included Computed Tomography (CT) and autopsy and highlighted the presence of two entrance “near contact” gunshot wounds on the left pectoral region. On the posterior chest area, one exit gunshot wound was found, as well as a retained projectile under the skin. Both bullet trajectories were intrathoracic. Death was certified due to hemorrhagic shock from cardiac and pulmonary lesions from SGW.

**Case 2:** A 64-year-old man was found dead on the kitchen floor of his house. The body was in a semi-prone right-side position, surrounded by blood. Body examination revealed three penetrating lesions to the head, two on the right parietal region and one on the opposite parietal region. A semi-automatic pistol (Beretta® mod. 950, .25 ACP) was found under the body. History revealed that the subject suffered from flaccid paraplegia and had family conflicts. Postmortem examination included CT and autopsy and highlighted the presence of two entrance wounds identified as “contact” gunshot injuries on the right parietal region. Moreover, on the left parietal region, one exit gunshot wound and a bone-lodged retained projectile were detected. The cause of death was attributed to brain lesions from SGW.

Multiple gunshot wounds are suggestive of homicide, especially when they are reported to the head. In these cases, accurate forensic and medicolegal investigations are fundamental to accurately differentiate and classify the death. In the reported cases, collected data and information allowed us to classify the deaths as suicide. Suicides were supported by analysis of the circumstantial information, the type and characteristics of the weapon, the location and the characteristics of the gunshot wounds, the study of damaged organs and the evaluation of the decedent’s “ability to act” voluntarily in the survival time.<sup>1</sup> Moreover, a literature review of cases of SGW was performed, revealing interesting common features. Finally, both of the discussed cases confirmed that most suicides involving a weapon in Italy occur at home, involve older male subjects, are firearm-related (short weapons, most frequently), and involve the head and chest regions.<sup>2</sup>

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### Self-Inflicted Gunshot Wounds, Suicide, Forensic Pathology

## H62 Disability-Related Road Traffic Crashes Involving Children

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of disability in children caused by traffic accidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the awareness to eliminate the causes of accidents, to prevent future adults from being disabled, to plan training programs, and to make recommendations on preventive measures.

Traffic refers to people, animals, and vehicles that are moving along the roads. Road traffic accidents involve people and one or more vehicles that are stable or moving and result in death, injuries, or damage.<sup>1,2</sup> According to the World Health Organization (WHO), road traffic accidents are the leading cause of death due to injuries, the tenth cause of death in general, and the ninth cause of death due to burden of disease. In 1998, road traffic accidents caused 2.8% of all disabilities, while they were the leading cause of disabilities caused by injuries. Road traffic accidents are expected to be the third cause of death due to burden of disease in 2020.<sup>3</sup> The frequent exposure of children to trauma, violence, and abuse has required protective laws all over the world. All injuries that occur in an adult in a traffic accident can also occur in a child, but the locations of the primary lesions differ in children because their height is shorter than adults.<sup>4</sup> Although it is extremely dangerous for children to travel in the front seat without a seat belt, on a lap, or standing, parents still allow it. In such cases, it is inevitable that the child will hit the windshield as a result of sudden braking or be injured or die from being trapped between the front panel and the parent's body.<sup>5</sup> Seat belts that ensure the safety of adults are not suitable for children because they are physically smaller. In this context, the use of child car seats has been compulsory since 2000 in some European countries (Switzerland, Sweden, France, Norway, Portugal, etc.).<sup>6</sup> In Turkey, according to the Highway Traffic Law numbered 2918, as of June 1, 2010, the use of child car seat systems suitable for children under 150cm and less than 36kg in the vehicle is obligatory; this regulation also prohibits children under 10 years of age to be transported in the front seat.<sup>4</sup>

Prospective examination of 106 pediatric victims of road traffic accidents evaluated by the Department of Forensic Medicine, Cukurova University, between July 1, 2019, and July 1, 2020, was undertaken to determine the presence and severity of any resulting disabilities. The cases were reviewed in terms of age, gender, accident type, affected body parts, surgical interventions, and lesions to allow measurement of disabilities and the rate of disabilities. The data gathered was statistically analyzed.

Among the 106 cases, the youngest case was a 2-year-old, 17 were 10 years of age and younger, 89 were between the ages of 11–17 years. Eighty-nine cases (74.5%) were male and 17 (24.5%) were female. Seventeen cases were injured inside the vehicle, 49 cases on motorcycles, 9 cases on bicycles, and 31 cases as pedestrians. Eighteen of the cases (16.9%) were motorcycle drivers who didn't have a legal driving license. Fifty-eight percent of cases had a head and neck injury. Twenty cases (18.8%) underwent surgical treatment. In this study, the rate of disability in 45.2% of the cases was found to be between 10%–30%.

Children are the future of the world and it is our duty to protect them. In childhood motorcycle accidents, male adolescents were more involved. Road traffic accidents not only cause deaths and property damage, but also serious illness and disability. Larger studies are needed to determine preventive measures to eliminate cause of accidents, to prevent future adults from being disabled, to plan training programs, and to make recommendations on preventive measures.

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### Childhood, Traffic Accidents, Disability

## H63 Widespread Polyvisceral Vascular Angiectasias in an Electrocution Victim: A Case Report

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**Learning Overview:** The goal of this presentation is to show that the combination of particular pathological mechanisms may have been the cause of angiectasias. This histological finding is particularly rare and can provide a valuable aid to determine the true cause of death in victims where electrocution is suspected, but there are no detectable body marks or circumstantial data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that, from a forensic point of view, such a case demonstrates once again that a thorough forensic investigation is mandatory in order to reach a correct cause of death by electrocution. In particular, a histological analysis is always required to highlight infrequent but useful findings.

The aim of this report is to present a case of electrocution death, confirmed by skin electric marks, circumstances, and histological findings showing widespread polyvisceral vascular angiectasias. These findings have rarely been described in literature.

A 57-year-old male, in good health and without any known cardiovascular disease, was hit in the chest by a cable carrying 230 volts while he was working with a colleague inside an electrical substation. The colleague heard a sudden gasp and saw the man slumped on the ground. As the colleague tried to drag the man out of the cabin, the colleague was also hit by the current. The colleague was finally able to remove the man from the location of the incident by pushing him with his feet.

Evidence showed the man was working in the absence of appropriate personal protective equipment and before the fatal event was in a kneeling position, working on the lower part of an electrical cabinet. Emergency medical services came quickly, but the doctor could only ascertain that the death was due to “electric shock.”

The external examination revealed fixed, red-wine-colored hypostases in the posterior regions, conjunctival injection, and cyanosis of the face and the neck. On the left parasternal line, at the height of the sternal handlebar, there was a purplish excoriation with a reddish parchment-like halo, slightly raised, measuring 4.5 x 3cm, compatible with an electrical mark. On the lower limbs, two slightly curvilinear, brown-black, hard, and parchment-like excoriations were also evident, about 10cm to the right and 6cm to the left, compatible with exit lesions.

At the autopsy, numerous sub-pleural petechiae were found bilaterally. The lungs were heavily congested and markedly edematous. Polyvisceral congestion was observed. There were no other macroscopic pathological findings.

Histological examinations documented widespread polyvisceral vascular angiectasia of all the organs. The lungs showed edema, focal atelectasis, vascular congestion, rupture of the septa, and intra-alveolar hemorrhage. The brain parenchyma was markedly edematous. The electrical mark had widespread dermal homogenization with compaction/elongation of the fibroblastic cells and the disappearance of nuclear and cytoplasmic detail. The exit lesions showed large areas of ulceration with disruption of the epidermis and neutrophil infiltrations.

Investigations concluded that the cause of death was an acute cardiorespiratory failure secondary to electrocution.

It is well known that electric current flows along a circuit loop with minimal electric resistance. In the living body, the electric resistance of blood, neural, and muscular tissue is relatively low. According to the scientific literature, electrocution causes endothelial lesions; specifically, it causes the formation of pores in the membranes of the cell bilayer due to a strong increase in transmembrane potentials.<sup>1</sup> Other studies explain how the passage of electricity through tissues causes the release of free radicals, which would damage the tissues themselves, in particular the heart and vessels.<sup>2</sup> Further studies show that the electrical damage causes a complete loss of endothelial cells, and that the muscle fibers of the media are particularly sensitive to electrical current. As a result, the vessels lose their elasticity and aneurysms could form.<sup>3</sup>

The combination of these pathological mechanisms may have been the cause of angiectasias. This histological finding is particularly rare and can provide a valuable aid to determine the true cause of death in victims where electrocution is suspected, but there are no detectable body marks or circumstantial data.

To conclude, from a forensic point of view, such a case demonstrates once again that a thorough forensic investigation is mandatory in order to find that the correct cause of death was by electrocution. In particular, the histological analysis is always required to highlight infrequent but useful findings.

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### Electrocution Death, Vascular Dysfunction, Angiectasias

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## H64 Acute Progression of Traumatic Diaphragmatic Laceration With Gastric Herniation in the Context of Underlying Chronic Disease

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**Learning Overview:** The goal of this presentation is to explain the value of maintaining high clinical suspicion for the pathological progression of a trans-diaphragmatic gastric hernia following trauma, especially in the context of significant comorbidities. This presentation will also highlight the importance of forensic autopsy performance for quality control and quality assurance purposes, with the ultimate goal of preventing future deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a retrospective analysis of clinical decisions that provide insight and useful instruction for future medical and surgical care. This report seeks to provide context for one such case in which a suspected trans-diaphragmatic gastric herniation, discovered following acute trauma involving a patient with underlying chronic disease, was left untreated, leading to the patient's death within days of a Motor Vehicle Accident (MVA) in which the decedent had suffered "non-life-threatening" injuries.

An 84-year-old man with hypertension, atherosclerotic cardiovascular disease, diabetes, and emphysema died days after being involved in a head-on MVA traveling at 55 miles per hour. The man was the restrained driver of one of the vehicles. He was found awake and alert at the scene. He was assessed by a trauma surgeon to have sustained only non-life-threatening injuries from the accident, including mild trauma of the frontal surface of the scalp with no evidence of intracranial hemorrhage. Because of his age and comorbidities, he was kept overnight in the hospital for observation. Early in the morning following the MVA, a trauma surgeon described the presence of a radiologically identified trans-diaphragmatic herniation of the patient's stomach into the left hemithorax, consistent with the radiology report upon admittance the previous night. The surgeon was uncertain as to the etiology of this herniation, suggesting that it could be either chronic, such as a hiatal hernia, or the result of acute trauma. Both the surgeon and radiologist favored a chronic process. Ultimately, the patient's diaphragmatic hernia was not attended to by the surgeon. Instead, a decision was made to observe the patient. After approximately 36 hours of hospitalization, the patient suddenly became bradycardic and died, despite efforts at resuscitation. A medicolegal autopsy was performed. This revealed a gaping, 10cm x 5cm acute, traumatic diaphragmatic laceration with significant stomach herniation, a left hemothorax with approximately 300mL of liquid blood, and marked compression of the left lung. Based on these findings, it was determined that the decedent had died from hypertensive and atherosclerotic cardiovascular disease with a contributing factor of complications of trauma, specifically a diaphragmatic laceration with gastric herniation leading to lung compression and eventual compromise.

### Forensic Pathology, Motor Vehicle Accident, Gastric Herniation

## H65 Cassowary Fatally Attacks Its Owner

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**Learning Overview:** The goal of this presentation is to illustrate the type of injuries inflicted by a large wild bird. Presented here is a systematic postmortem examination of the injuries caused by wild giant birds. Specific anatomic features of wild animals will serve as weapons either for predation or for defense. This presentation describes the lethal injuries a cassowary owner sustained after being attacked by one of his birds during an attempt to retrieve one of her eggs. The specific anatomy of the dagger-like second claw of the cassowary's second digit allows this animal to cause deadly sharp force trauma to vital parts of a human in an attack.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the challenges of safely keeping wild animals. When working or interacting with kept wild animals, the perception of the danger inherently associated with these animals may decrease with time, and humans are likely to take fewer safety measures when interacting with these wild, non-domesticated animals. This in return can lead to incidences that may end in death, as it does in this case. Attendees will also learn some anatomic features of wild large birds that serve as weapons and the type of resulting lesions that are inflicted during a lethal attack.

A 75-year-old Caucasian male was the owner of an exotic animal and bird farm. Among the bird species, he kept cassowaries, which are giant terrestrial omnivorous birds, related to ostriches and emus.

Rheas and kiwis are native to the tropics of Australia and New Guinea. In Florida, possession of cassowaries requires a permit from the Wildlife Commission, which classifies them as "Class II wildlife" and together with ostriches, they are known to be dangerous, representing the only two bird species of the Family *Casuariidae* known to have fatally attacked and killed humans. Some physical characteristics of cassowaries are their height (growing up to 1.90m), their weight (up to 60kg), their rather short but strong beaks, their keratinized head crest, as well as their three-digit feet. Each digit is equipped with a large sharp claw. The claw of the medial digit (digit II) is dagger-like, nearly 13cm in length, and serves as a primary weapon. Digit I is absent and the reduced wings exhibit spine-like quills. Although cassowaries cannot fly, they can run up to 50km/h and jump up to 1.5m high. When startled or provoked, they can inflict serious injuries by kicking in a forward and downward direction, pecking, barging, or head-butting, causing puncture wounds, lacerations, and even bone fractures. Attacks are mostly due to association of humans with food, or territorial reactions where humans are present in areas where the cassowary is feeding or areas where it was defending itself, its eggs, or chicks.

In the past, the owner had been injured by one of the large birds with wounds to his leg. Normally, when entering the pen of a pair of cassowaries, he would hold a "shock stick" and had a second person with him. On that day and for unknown reasons, he was alone and had entered the pen to retrieve one of the female's eggs. The eggs sell for a substantial amount of money to other breeders. When the male cassowary saw his attempt to retrieve an egg, he attacked. During the attack, the owner had fallen down but was able to call for help; by the time he was pulled to safety, he had sustained numerous injuries and had lost a substantial amount of blood. He was rushed to the hospital.

There, the most significant injury identified was a brachial artery laceration with avulsion of the profunda brachii artery that was repaired along with other superficial to deep skin lacerations. The patient did not survive his injuries and his death was reported to the medical examiner in accordance with Florida Statute 406.

At autopsy, multiple, irregular, linear, and curvilinear abrasions, superficial punctures, and lacerations were randomly distributed on the face, neck, anterior and posterior trunk, arms, and legs. Numerous injuries were sutured or stapled shut. Long sutured or stapled surgical incisions were across the chest (clam shell incision) extending from right to left axilla, on the right upper arm, and the mid abdomen. The left lung lobe was completely collapsed, airless and hemorrhagic. Additional findings unrelated to the incident were aortic atherosclerosis with mural thrombi, moderate nephrosclerosis, and prostatic nodular hyperplasia.

The most probable cause of death was numerous sustained injuries in attack by cassowary and the manner of death was an accident.

The patterned injuries are consistent with the attacking behavior of cassowaries where the "daggered-claw" most likely caused the right arm injury and the brachial laceration injury of the chest in a forward kick of the bird followed by stomping or barging, as well as pecking of the victim. The latter three likely caused the numerous more superficial punctures and lacerations. After the victim fell to the ground, the attack continued for an unknown period of time until his outcries alerted his wife who entered the enclosure and repelled the female cassowary with a "stun gun."

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### Cassowary, Wild Animal Fatalities, Sharp Force Trauma

## H66 A Fatality by Cattle Hooves in an Elderly Cowherder: Is There a Specific Pattern of Hoof Injury?

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 hoof injuries by hoof-bearing animals that proved fatal to a cowherder.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating awareness of specific forms of injury by cattle hooves that may be of medicolegal significance if such a case is found in deserted places with no witness to the incident. Presented here is a study highlighting such an incident that is rarely mentioned in the available literature.

Cattle are socioeconomically essential creatures that, due to their size and power, can cause injury and death.<sup>1</sup> In spite of the large number of recognized animal/livestock injuries, little is known about factors and activities that raise or decline the danger of injury. Farm animals can bite, kick, gore, trample, fall on, step on, knock down, crush, pin between other animals or farm structures, peck, scratch, throw, buck, drag, ram, or butt.<sup>1,2</sup> Horses and cattle, rather than any single type of agricultural machinery, are reported to be the leading cause of injury.<sup>3</sup> Cattle have about 360-degree panoramic vision. A swift movement at the rear of cattle may “spook” them. They have exceptionally sensitive hearing and can sense sounds that human ears are unable to hear. Loud noises scare animals, and research shows that high-frequency sounds in fact hurt their ears. These features explain why animals are often frisky and balky, mainly in unknown environments.

In the present case, a 70-year-old cowherder was herding his cattle in open land for grazing. He had been doing this for many years and was well versed with the route and animal behavior. Though he was old, he was in good health and had a loud voice that was sufficient for the cattle to obey his commands. He would always carry a bamboo stick and an axe for personal protection and also for herding the cattle. On the day of the incidence, as usual, he was herding a group of about ten cattle in open land near his farm. When he didn't returned home, family members searched for him. He was subsequently found lying unconscious near some boulders with his head showing injuries from which blood had splattered onto some surrounding boulders. The cattle were seen calmly standing beside him without any aggressive gestures. His stick and axe were lying beside him without any evidence of blood staining. He was taken to the tertiary health center where he was declared dead.

On autopsy examination, there were approximately four “C”-shaped lacerated wounds present over the frontoparietal region of the head; 8 to 10cm in length and extending to the bone. There was underlying subscalp hematoma with depressed comminuted fractures of the skull that were roughly oval in shape. On investigation, one of the cattle who was new to the herd had blood stains over its right front hoof. Also, there were no signs of any other foreign objects or any human interference at the site of the incidence. It was concluded that the suspected animal was responsible for the fatal injuries to the elderly man.

This presentation will discuss the importance of having knowledge regarding how these injuries can occur and lead to death.

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### Fatality, Cattle, Hoof Injury

## H67 A Quantitative Investigation of Diatom Dispersion in Lung Tissue of Confirmed Drowning Incidents

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**Learning Overview:** The goal of this presentation is to provide additional information about the mechanism and dispersion of diatom inhalation during a drowning event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how a quantitative method of diatom analysis is a sufficient tool for the diagnosis of drowning and how microorganisms are distributed within lung tissue upon inhalation of a drowning medium.

According to the World Health Organization (WHO) in 2020, drowning is the third most-common cause of unintentional injury-related death worldwide. A reliable identification of death by drowning can be subtle and not always possible, if certain drowning signs like foam cones, emphysema aquosum, or Paltauf's spots are absent. Therefore, diagnoses are often arrived at by combining the findings from autopsy, toxicological tests, and fine-structural examination of internal organs. A quantitative investigation of diatoms present in lung tissue serves as another good indicator to confirm a drowning. Based on the consideration that diatoms are present in almost every natural water body and are incorporated when water is inhaled as a result of the respiratory reflex, their presence in lung tissue and other internal organs is thus proposed as a sign of death by drowning. According to previous research, however, a diagnosis of drowning by the mere presence of diatoms in peripheral organs remains controversial due to the high inaccuracy and susceptibility for contamination effects.

While the classical diatom test focuses on qualitative analysis per light microscopy, a recently developed method utilizes a quantitative assessment by comparing the concentration of diatoms between lung tissue and the corresponding drowning medium, which allows for the calculation of an L/D value. The adaption of this technique (Microwave Digestion-Vacuum Filtration-Automated Scanning Electron Microscopy) achieves a maximum recovery of diatoms, ensures an easy identification with high resolution, and was optimized and validated for implementation. As part of routine autopsies of drowned individuals, tissue samples of lung, liver, and kidney as well as the drowning medium were taken as evidence for analysis. To determine a ratio between tissue and drowning medium, a conventional acid digestion, automated filtration, and quantification by scanning electron microscopy was applied. For previous case studies, lung tissue samples were taken from the upper left tip of the lung, the area of lowest inhalation pressure, due to the assumption that the probability of a possible drowning event was highest if diatoms were present there. It is unclear whether different parts of lung tissue would display different diatom concentrations. To investigate regional differences in general, and to determine which part of the lung is best qualified for valid results, the diatom ratio of multiple sampling sites within the lungs were compared. Seven different regions allocated across both pulmonary lobes of three confirmed drowning cases were examined to obtain a broader understanding of the mechanisms of diatom inhalation and ultimately the replicability, accuracy, and precision of this technique.

### Drowning, Diatom Test, Scanning Electron Microscopy

## H68 The “Key” Element in a Case of False Decapitation of a Missing Person: A Case Report and Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will understand the role of forensic investigations in missing persons cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting a case of “false” decapitation of a missing person whose body was found in an advanced state of decomposition.

In Italy, according to data from the Interior Ministry, about 24 people per day disappear, of which 5 are minors. In 2016, 189,110 people disappeared in Italy, of which 43,665 were never traced. In the analysis of elderly people who have gone missing, the main cause is disorientation due to cognitive impairment, as in the case of Alzheimer’s disease. With young adults, the main reason is voluntary removal. With regard to minors, the phenomenon mainly concerns foreigners and is connected to trafficking of young slaves induced into prostitution, as well as for the sale of organs. In several cases, the missing people are later found dead following recognition or through DNA comparisons.

The coroner is responsible for identifying the body; however, identification is not easy in bodies in an advanced state of decomposition with alteration of their facial traits and without documents. Personal identification is an often complex, requiring comparison between premortem photographs, descriptions, X-rays, dental records, or biological material against that obtained at postmortem examination.

This is a case of a Caucasian unidentified body. The body was decapitated, aged between 25–35 years, found under the slopes of a hill in the province of Catanzaro. During the judicial inspection, the head appeared detached from the rest of the body, an element that led the investigators to suspect a beheading. The body was in an impenetrable cliff that was accessed through an underlying street. The body was in an advanced state of decomposition with skeletonization of the head that was located about six meters from the body. The body was immersed in large quantities of garbage that hid it. The analysis of the garments showed the presence of a number of keys inside a pocket with large lacerations. An investigation of the clothes was performed. At autopsy, the analysis of putrefaction, environmental climatic conditions, and cadaveric temperature estimated the death to about 120 days prior to the body being found.

At autopsy, entomological elements of *Lucilia sericata* were found externally with eggs, larvae, and adult specimens of flies. The injuries reported, all having characteristics of vitality, were compatible with a fall from a great height (crushing of the thoracic cage, widespread fractures in the cervical and thoracic spine). The injury was compatible with a violent impact on the posterior cervical and thoracic surface. The head, subsequently analyzed with appropriate histological examination, did not show the common signs of vitality of bone stumps. In particular, postmortem linear defects compatible with animal bites were found on the bone. This analysis showed that the beheading was linked to animal scavenging of the body, facilitated by trauma of the cervical spine (loss of skeletal continuity) and putrefactive changes. After clarifying the cause of death through collaboration with investigators, teeth and the genetic profile of the subject were obtained. Subsequently, a compatible subject was identified among a list of missing people in the neighboring areas. In particular, the keys played a crucial role in identifying the subject’s home. The comparative genetic analysis confirmed the identity of the missing person. Due to this case, a standardized method of cataloging personal effects is proposed with the help of at least three operators to identify objects belonging to an unidentified subject on the scene, by obtaining details of garments or objects that can subsequently assume a key role in solving the case.

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**Forensic Sciences, Decapitation, Missing Person**





## H69 An Unusual Presentation of Asphyxia Due to Choking

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**Learning Overview:** Attendees will learn that the autopsy alone for postmortem analysis is only part of the death investigation. This unusual case of asphyxia emphasizes the collaborative input of investigators, detectives, medical examiners, and other health care professionals to determine the cause and manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an unusual circumstance in which death was caused by asphyxia due to choking. A detailed death investigation utilizing a team of death investigators were essential to fully explain this death. This case is presented so that similar cases might be compared as well as to highlight the importance of gathering all the facts to explain unusual circumstances surrounding autopsy findings with an unusual presentation.

The decedent was a 32-year-old male with a non-contributory past medical history. He entered a publicly hosted insect (bug) eating competition in which the prize was an exotic reptile. The contest rules stated that contestants were to consume as many female pastel superworms, female orange belly hornworms, female graphite sire discoid cockroaches, or male lesser crickets in the time constraints of four minutes with one hour between each segment time. Water was allowed during the competition. The decedent won the discoid cockroach eating competition segment, began vomiting, and eventually became unresponsive and was later pronounced deceased at a hospital.

Postmortem examination revealed evidence of choking and aspiration. Scleral hemorrhages were appreciated. Arthropod body parts were aggregated in the trachea, main stem bronchus, and bilateral segmented bronchi. Petechial hemorrhages were scattered over the mucosal surface of the epiglottis and larynx. The stomach contained partial and whole cockroaches, segmented larvae, and caterpillars.

Due to the possibility of an anaphylactic reaction secondary to consuming insects, specialized testing for immunoglobulin E and trypsin were conducted and reported within normal limits.

The presence of insects in these locations is not enough to determine cause of death. Careful consideration and laboratory testing were taken to rule out allergic or anaphylactic response to the presence of insects versus aspiration of stomach contents (insects) causing asphyxia due to choking.

The death investigation was extremely important because it allowed the medical examiner to explain why insect parts were present in the airway and alimentary tract. It further helped to identify the types of insects that were present. The responding detective to the scene was able to obtain the information on the competition and the events that led to the death. The health care professionals (emergency medical services, emergency room doctors, nurses, staff) provided information on the medical events that occurred from their arrival at the scene, transportation of the decedent to the hospital, and emergency room care. Finally, the medical examiner conducted the postmortem examination correlating the autopsy findings with the circumstances surrounding the death. All the individuals involved in this case worked together in a multidisciplinary fashion to provide a complete picture of the decedent's unusual demise.

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**Asphyxia, Choking, Forensic Pathology**

## H70 A Lethal Fat Embolism Syndrome (FES) Related to Spine Surgery: A Presentation of Two Clinical Case Reports

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**Learning Overview:** After attending this presentation, attendees will understand the importance of a scrupulous autopsy to define the causes of death following spinal surgery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community due to the rarity of reports about FES related to neurosurgical procedures. The autopsy technique and the methodological approach to histological investigations will also impact the forensic science community.

FES is a clinical condition characterized by the obstruction of small vessels by fat emboli; it presents with neurological, respiratory, hematological, and cutaneous manifestations. Its death rate is variable depending on the number of vessels and organ systems involved. It generally occurs after long bone fracture (especially femur fractures). Spinal arthrodesis is often needed to stabilize the spinal column after different spinal disorders like traumatic fractures, idiopathic disease (such as scoliosis), metastatic tumor, infection, or degenerative disorder. In the literature, there are only four cases reported of FES-related deaths occurring after spinal elective surgery; it is considered a rare complication of this procedure. Although spinal fusion surgery can be associated with serious and various complications, perioperative mortality is generally considered a rare event; the death rate reported in the literature is about 0.13% of all procedures. Two cases of lethal FES related to spinal fusion surgery are presented.

**Case 1:** A 64-year-old woman with chronic obstructive pulmonary disease, atrial fibrillation, and chronic kidney disease was admitted to a private surgical clinic to undergo posterior spinal fusion surgery for degenerative scoliosis presenting with lumbar canal stenosis and neurological deficit. All procedures were performed well with surgical screws and side bar correctly positioned in cemented vertebrae. After three hours of surgery, the patient had a significant desaturation that progressed to hypoxemia and severe bradycardia, requiring resuscitation maneuvers. Resuscitation failed. At autopsy, no significant pathology was seen except for polyvisceral congestion and pulmonary edema, with several Cardiopulmonary Resuscitation (CPR) - related fractured ribs. No blood clots or emboli were found in the pulmonary arteries or heart chambers. Microscopic examination revealed the presence of optically empty vacuoles that interrupted red blood cells that were pushed peripherally in the vessels of the lung, brain, and kidneys. Immunohistochemical analysis (using anti-CD16 and anti-fibrinogen antibodies) permitted FES to be ruled as the cause of death.

**Case 2:** A 51-year-old healthy woman accessed a private surgical clinic for an elective spinal arthrodesis due to progressive and symptomatic idiopathic right convex scoliosis. The surgical procedures were performed without complications. She was briefly monitored in a postanesthesia room and subsequently discharged to the surgical ward in stable condition. Two hours later, the patient was found unconscious and in cardiac arrest by the nurses. Despite CPR maneuvers, the patient was declared dead. Autopsy results were not significant: polyvisceral congestion and about 600cc of blood collection in the peritoneal cavity without any macroscopic arterial or venous vessel damage were found. Ventral and dorsal study of the spinal column in conjunction with radiographic images confirmed the correct position of screws and bars. Microscopic examination found the presence of optically empty vacuoles involving a large number of pulmonary vessels, with blood cells displaced peripherally. Positivity of Sudan III and Oil Red O stains revealed that the cause of death was a massive (Grade III of Falzi's semiquantitative morphometry) FES.

In conclusion, two FES-related deaths after posterior columnar stabilization are presented. Despite it being a rare complication of this type of procedure and that the mortality rate of elective posterior spinal fusion is low (0.13%), forensic pathologists should be aware of FES in assessing these cases.

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### FES, Spine Surgery, Unexpected Death

## H71 An Unusual Hospital Asphyxiation: Death From the Incorrect Administration of Nitrous Oxide (N<sub>2</sub>O)

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**Learning Overview:** The goal of this presentation is to describe an unusual case of death due to the incorrect administration of N<sub>2</sub>O during anesthesia. Despite a first autopsy that established death due to myocardial infarction, the effective cause was diagnosed during a second necroscopic examination. The use of immunohistochemical markers has been important in detecting hypoxic damage in tissues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a rare case of death due to the incorrect administration of N<sub>2</sub>O during anesthesia. The role of immunohistochemistry in the investigation of hypoxic damage is also emphasized.

N<sub>2</sub>O is the most commonly used inhalation anesthetic in dentistry.<sup>1</sup> However, the safety and efficacy of N<sub>2</sub>O has been questioned; indeed, N<sub>2</sub>O is no longer used in anesthesia.<sup>2</sup> N<sub>2</sub>O produces moderate analgesia at subanesthetic concentrations and also an anxiolytic effect.<sup>3</sup> Anesthetic concentrations are 50%–67%; higher concentrations cause asphyxiation, indeed, the administration of N<sub>2</sub>O is associated with hypoventilation.<sup>4</sup> Because of the sedative and euphoric properties of the N<sub>2</sub>O, it is often used for recreational use.<sup>5</sup>

Accidental deaths are mainly associated with recreational uses of N<sub>2</sub>O. In the literature, there are rare reports of accidental inhalation at work or due to its incorrect administration to hospitalized patients during anesthesia.<sup>6,7</sup> Often, asphyxia from N<sub>2</sub>O as a cause of death is attributed thanks to the analysis of the crime scene, while the autopsy plays a marginal role. Generally, the autopsy shows only generic signs of asphyxia, but nothing that can lead directly to N<sub>2</sub>O intoxication, such as histopathological examinations with standard Hematoxylin-Eosin (H&E) staining.<sup>8</sup> Immunohistochemical research of hypoxic markers and toxicological analysis are more useful.<sup>9,10</sup>

This case report is that of a 70-year-old man who underwent endovascular prosthesis placement surgery to treat an aneurysm of the left common iliac artery. The surgery was performed under local anesthesia. During the operation, an oxygen mask was attached to a ventilator. Suddenly, the patient became unconscious and there was rapid oxygen desaturation; the anesthesiologist subsequently switched to endotracheal intubation. Thereafter, the patient went into cardiac arrest and died.

The first autopsy diagnosed recent myocardial infarction of the left ventricle, cerebral and pulmonary edema, splenic infarction, multi-visceral congestion, atherosclerosis of the aorta, and sub-occlusion of the coronary arteries (about 60% of the lumen). Histological examination confirmed these findings. The cause of the death was acute pulmonary edema due to recent myocardial infarction of the left ventricle.

Subsequently, the operating room was checked by technicians and an inversion of the oxygen and N<sub>2</sub>O pipes was found. This had occurred during its building, so that the patients breathed N<sub>2</sub>O instead of oxygen.

Two months later, a new autopsy was performed by a forensic pathologist on the same body. Surprisingly, the second examination found that the coronary arteries were not sectioned and analyzed, although this was described in the first autopsy report. They sampled organs again to perform further histology and immunohistochemical investigations. Histological samples from the first autopsy were re-examined and other slides were stained with H&E staining—immunohistochemistry for E-selectin (CD62E-382), P-selectin (CD62P), monoclonal mouse anti-human (DAKO), and HIF 1- $\alpha$ .

Study of the slides from both autopsies revealed no coronary artery stenosis. The cardiac myofibers showed diffuse interstitial neutrophilic infiltration, without signs of necrosis, with the presence of contraction bands. Splenic infarction was not detected. Examination of the brain tissue revealed loss of Purkinje cells, suggesting hypoxic damage. Immunohistochemical staining showed that in the heart, HIF 1- $\alpha$  was expressed in the myocardial nucleus areas showing signs of reperfusion. HIF 1- $\alpha$  was also expressed in areas close to hemorrhagic extravasation and in areas with fibrotic replacement.

The P-selectin revealed positivity of platelets aggregate, expressed widely in the areas of hemorrhagic extravasation. E-selectin was expressed in the same areas, but less intensely. In the lung, P-selectin was expressed widely in medium vessels of the emphysematous lungs. Hemorrhagic areas were also positive for P-selectin. The findings suggest that all myocardial tissue had suffered from a lack of oxygen perfusion. Even the brain had suffered hypoxic damage.

So, combining the autopsy findings as well as the investigations carried out by the technicians on the operating theatre and ventilator, the truth was discovered. The real cause of death was intoxication by N<sub>2</sub>O, with acute asphyxiation due to hypoxia.

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## **Nitrous Oxide, Hypoxic Damage, Anesthetic Inhalation**

## H72 Exsanguination Due to Urethral Laceration

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**Learning Overview:** The goal of this presentation is to report a case of urethral laceration due to Foley catheter manipulation leading to hemorrhage and eventual death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the approach and findings related to deaths due to urethral injury from Foley catheter manipulation.

A Foley catheter is frequently used in medical settings to facilitate bladder emptying. There are multiple Foley catheter-related complications, including urinary tract infections and Foley catheter related genitourinary trauma.<sup>1-3</sup> While the death investigation of urinary tract infection related to Foley catheter use is likely to be uncomplicated, the death investigation of urethral laceration and hemorrhage due to Foley catheter manipulation is not well documented.

This is a case of a 65-year-old White male with a complex medical history who presented to a local hospital from a rehabilitation facility. The decedent was undergoing an attempted change of his chronic indwelling Foley catheter when the patient experienced pain. The initial Foley catheter was removed and a second insertion attempt was performed. The decedent continued to experience pain, and there was a significant amount of urethral hemorrhage. The decedent was transported to a hospital and surgical intervention was performed, where evidence of significant urethral trauma in the bulbous urethra with active bleeding as well as active bleeding from the prostate was observed. The decedent experienced intraoperative hypotension and tachycardia and postoperative respiratory failure.

In performing the postmortem examination, careful considerations were made in order to best visualize the extent of the urethral injury suffered by the decedent. It was decided that the best approach in this case would be a cross section of the decedent's genitalia in the sagittal plane along the urethra. This revealed a laceration of the proximal urethra, superficial abrasion of the mucosa at the junction of the bladder and prostate, and superficial abrasions of the bladder mucosa. The cause of death was urethral laceration following a Foley catheter manipulation and the manner of death was certified as accidental.

Catheter-related trauma is a known complication of Foley catheter placement.<sup>1-3</sup> While there are multiple reports about the rate of Foley catheter-related trauma and associated hospital course due to said trauma, the number of deaths due to Foley catheter-related trauma is unclear.<sup>4</sup> The literature search on fatalities related to Foley catheter-related death yielded case reports of bladder rupture from Foley catheter insertion, leading to death.<sup>4,5</sup> However, these cases are extremely rare and other reports of death due to hemorrhage resulting from urethral laceration due to Foley catheter manipulation were not found.

In cases where there was an internal injury due to Foley catheter manipulation, external examination of the genitalia is not sufficient. The length of the urethra going into the bladder should be exposed to examine the extent and the exact location of the injury.

This case is a rare example of complications of Foley catheter-related trauma that led to death. This case illustrates careful considerations forensic pathologists need to make when handling cases where there is a possibility of injury from the usage of Foley catheters that may have significant impact in determining the cause and manner of death.

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### Foley Catheter, Urethral Laceration, Urethral Injury

## H73 Neuropathologic Findings in Fatal Silicone Embolism Syndrome: Potential Contributions to Cause of Death

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**Learning Overview:** After attending this presentation, attendees will better understand fatal silicone embolism syndrome when approaching forensic autopsies. The goal of this presentation is to highlight the neuropathologic findings seen in systemic silicone embolism syndrome, which has been seldom described in the literature. After attending this presentation, attendees will better understand the pathophysiologic mechanisms involved in fatal silicone embolism syndrome and how to appropriately assign manner of death in these particular cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing to light the various neuropathologic findings seen in fatal silicone embolism syndrome, increasing awareness of this uncommon diagnosis, bringing forth the reasoning behind accurate manner of death reporting for these cases in the forensic arena, and defining both the well-known acute and less-understood long-term sequelae of this pathology.

The illegal or unregulated use of liquid silicone for cosmetic purposes, including breast and buttock augmentation, often injected subcutaneously by non-medical personnel at in-home “pumping parties” has been well-described in the medical literature as potentially fatal. Systemic silicone embolism syndrome shares many clinical similarities with fat embolism syndrome, and both may have fatal outcomes. Recent injections tend to present with respiratory distress, chest pain, nausea, and fever, followed by loss of consciousness and death within hours to days. Alternatively, some sequelae may appear many years later and reflect profound multiorgan complications, in particular chronic renal failure with calciphylaxis. General histopathologic findings in autopsy cases, whether deaths are recent or delayed, comprise intravascular rounded filling defects that do not stain with fat stains, such as Oil-Red-O. Despite the recognition of silicone emboli in lungs and other organs, there is a paucity of literature regarding involvement of the central nervous system. Herein, the experience of the New York City Office of the Chief Medical Examiner’s (NYC OCME) over a period of 12 years in which death certification included silicone embolism as a cause of or contributing factor to death is reported.

Thirteen such cases were identified in the NYC OCME files (6 Females [F] and 7 Transgender Females [TGF]; age range, 22 to 75 years). Sources of silicone exposure were related to breast implant (with non-traumatic rupture) ( $n=1$ ) and subcutaneous injection to buttocks, breasts, and/or face ( $n=12$ ). The intervals from exposure to death ranged from less than an hour up to 30 years, but were not always known with complete certainty. At autopsy, systemic emboli of silicone material were noted in all cases, except for the ruptured breast implant, which showed silicone pulmonary emboli. The organs most usually affected were the lungs and kidneys, with the brain, liver, heart, and spleen also often involved. Among the cases with longer intervals following exposure, reactive changes including granulomatous inflammation of the lungs, kidneys, and liver, with pronounced involvement of prior subcutaneous injection sites by both granulomas and fat necrosis. Of note, in three of the four with a long interval of silicone exposure and chronic renal failure, calciphylaxis (the deposition of calcium in skin and soft tissue sites such as buttocks, breasts, hips, and perineum) was a prominent feature.

Of the 13 cases, 7 cases had formal neuropathologic consultation and 1 of the cases submitted brain for histology independently. Of these 8 cases with histology performed on brain tissue, 4 showed intravascular vacuoles and 5 of the cases showed ischemic changes, including acute neuronal injury (2) and axonal swelling (3); 1 of the cases with axonal injury also showed a microscopic infarct. Two cases had no histopathologic changes. A minority of cases ( $n=2$ ) had associated petechial hemorrhages in the white matter, highly reminiscent of those seen in fat embolism syndrome. One of these cases also showed patchy areas of necrosis.

With regard to cause of death, 11 were attributed directly to systemic silicone embolization; 1 case had a confounding picture, including drug use disorder history and terminal hospitalization; the other case was likewise complex due to acquired immune deficiency syndrome, although silicone embolization was considered contributory because of infected ulcerated buttock lesions (injection sites). Of note, only 1 of the 11 systemic silicone embolization cases had a patent foramen ovale listed as a contributory factor.

With regard to manner of death, 5 of the 13 were deemed undetermined, 6 were homicides, and 2 were therapeutic complications. Those of undetermined manner had incomplete data regarding the individual performing the injection (i.e., self, another person, or both). Homicide cases were clearly those in which the injection was performed by another person, all of which were unlicensed, non-medical personnel. Of the 2 therapeutic complications, one was due to a non-traumatic ruptured silicone breast implant, while the other case had limited retained medical records at the time of this retrospective analysis.

In summary, clandestine use of liquid silicone for cosmetic purposes has acute as well as long-term effects, including on the central nervous system. Moreover, because of its unique physicochemical properties, it does not require an overt left-to-right shunt to gain access to arterial territories, leading to fatal systemic vascular occlusions.

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### Silicone, Embolism, Neuropathology

## H74 A Case of Death Due to Asphyxia From the Use of Mechanical Restraints in a Hospital Environment: Always an Accidental Death?

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**Learning Overview:** After attending this presentation, attendees will understand the risk linked to the use of mechanical restraints in psychiatric patients.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the need for surveillance in order to prevent the use of illegal restraints.

Asphyxia is due to an impediment to the inflow of air into the respiratory tract.<sup>1</sup> Strangulation is the compression of the neck by using a lacing or other similar tool. The first factor involved is the nervous one, determined by the compression and stimulation of the vagus nerve. Death by asphyxia can occur with suicidal, homicidal, or accidental manner. Sometimes the difference between the three manners of death is not clear.

The death of a boy suffering from Down syndrome found dead in a psychiatric clinic between the bed and the floor of the room in which he was hospitalized is reported. The investigators carried out a judicial inspection of the clinic. The scene was examined by investigating the use of restraints in the other rooms of the nursing home and on the other patients. An autopsy was performed with analysis of the injuries on the body and measurement of the chest diameter. The investigators seized and analyzed the mechanical restraints used; they measured its dimensions and reproduced its closure. The data were analyzed and compared.

The analysis of the scene showed the presence of a mechanical restraint that kept the boy tied to the bed. The restraint was found around the boy's thorax and neck, and it caused a constrictive mechanical action on the anatomical areas described. The tool used was a restraint belt 115cm long and 12.5cm wide. The belt was not elastic and it had buttonholes; outside the buttonholes were two other restraining tools with a plastic closure and a special metal device with a magnetic closure. The restraints were photographed and analyzed at autopsy. In particular, the compatibility of the metal device with a magnetic closure was assessed and ascertained. It was also established that the metallic device could only be opened by using a magnet that presented a specific key ring of green color. Thus, the restraining belt was further closed through the metal device and by a magnetic closure. Therefore, it was clear that the use of restraints had caused the boy's death by strangulation. In particular, the forensic investigations showed that all the patients of the clinic were tied by these tools during the night with no possibility of movement. In this case, unfortunately, probably in an attempt to get out of bed, the boy remained accidentally trapped in the mechanical restraint, thus causing strangulation with constriction of the chest. But is it really an accidental death?

In this case, an analysis was carried out regarding the role of health care personnel and the real need to use mechanical restraints. Restraint refers to the use of physical, pharmacological, and/or environmental tools aimed at limiting the ability of an individual to move.<sup>2</sup> Restraint has a prevalence of 15.8% in hospitals and 68.7% in nursing homes.<sup>3</sup> Fall prevention is the main reason restraint is used. However, some studies have shown the ineffectiveness of such systems in preventing falls; on the contrary, they often cause death by asphyxiation or trauma.<sup>4-6</sup> It is necessary to consider the use of mechanical restraints as an extraordinary and not ordinary intervention. The choice of the medium to be used should be considered in relation to the individual case and it must be limited in time. Every patient subjected to restraint measures must be carefully monitored by specialized personnel. Increased surveillance of the nurse/patient relationship is necessary to reduce the use of restraints. In this case, the lack of surveillance of patients subjected to restraint is highlighted. Therefore, the failure to monitor the patient and the unjustified application of these tools caused an avoidable death. The case suggests the need for more surveillance on the use of restraints on psychiatric patients. Avoiding improper use of restraints is important as is the guarantee of the right to physical and personal freedom.

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### Forensic Science, Mechanical Restraints, Hospital

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## H75 A Retrospective Review of Deaths in Custody Submitted for Medicolegal Autopsy at a Medical University in South Carolina (2012 to 2020)

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**Learning Overview:** After attending this presentation, attendees will have additional information regarding deaths in custody and the classification of cause and manner of death in these cases. The goals of this presentation are to contribute to the education of the public, media, law enforcement, and judiciary systems and to encourage discussion relating to how in-custody deaths are certified.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by supplementing the existing information available regarding deaths in custody and may be valuable in contributing to the public and political discourse/debate.

Recent high-profile events have brought the topic of in-custody deaths to the forefront of public discourse, raising questions related to patterns in the certification of death as well as the treatment of minorities in the criminal justice system.

A retrospective analysis of medicolegal cases referred to the Medical and Forensic Autopsy Division of the Department of Pathology and Laboratory Medicine at the Medical University of South Carolina (MUSC) from September 1, 2012, to August 31, 2020, was conducted. Search terms included “custody,” “restraint,” “police,” “jail,” “prison,” “law enforcement,” and “detention;” of the total 7,414 cases performed during that time period, 652 were identified as containing one or more of the search terms. The 652 cases were manually reviewed by two forensic pathologists and a total of 68 cases were identified as falling under the category of in-custody death, where custody was defined as “law enforcement official’s act of holding an accused or convicted person in criminal proceedings, beginning with the arrest of that person.” An additional 55 cases were identified where decedents were not in custody but died during interaction with or fleeing from law enforcement. The in-custody deaths were further categorized by demographic data, including decedent age, race, sex, manner of death, cause of death, and other variables such as incident site and the presence of drugs and alcohol.

Initial data indicated a preponderance of male decedents with 87% (59) male and 13% (9) female fatalities. There was an equal distribution of deaths between races with a Black:White ratio of 8:8.5. The average age of the decedent in custody was 37 years for females (range 23–58 years) and 44 years for males (range 24–67 years). The most common manner of death was natural with 25 (37%) deaths, followed by suicides and accidents at 13 (19%) deaths each, then homicides and undetermined manners of death at 8 (12%) each. One manner of death remained pending at the time of this abstract. Of the suicidal and accidental fatalities, the most common cause of death was hanging and drug intoxication, respectively. The majority of in-custody homicide fatalities included a component of restraint asphyxia whereas in-custody fatalities where the manner of death remained undetermined involved a variety of mechanisms, including restraint, blunt trauma, and history of seizure.

The data accumulated in this retrospective analysis will supplement existing information regarding deaths involving law enforcement and incarceration and may be valuable in contributing to the education of the public, media, law enforcement, and judiciary systems.

### **In-Custody Death, Autopsy, Forensic Pathology**



## H76 Acute Infectious Esophagitis: Three Cases With Variable Etiologies

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**Learning Overview:** After attending this presentation, attendees will: (1) appreciate the common and rare etiologies of acute infectious esophagitis; (2) compare and contrast the gross and histologic findings of acute infectious esophagitis; and (3) understand the risk factors and clinical outcomes for those with acute infectious esophagitis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting rare findings found at autopsy on decedents with acute infectious esophagitis.

Acute infectious esophagitis is a condition that is typically caused by viral or fungal infections. The most common cause of fungal esophagitis is *Candida* species, while herpes simplex virus and cytomegalovirus are the most common culprits of viral esophagitis.<sup>1</sup> Acute infectious esophagitis of bacterial etiology is exceptionally rare, particularly when caused by *Sarcina ventriculi*, a human pathogen discovered in 1842 that typically affects the gastrointestinal tract, with fewer than 20 cases reported.<sup>2</sup> The most common reported risk factors include immunosuppressive agents, diabetes, and long-term antibiotic use. Patients with infectious esophagitis will typically present with dysphagia, and it is classically diagnosed after performing an endoscopy exam and looking at biopsy samples histologically. Tissue cultures are also routinely performed to help identify the pathogen as well as hone in on more specific treatments. Gross and histologic findings can be variable, ranging from non-specific erythema and inflammation, to pathogen-specific discoveries like diffuse white plaques (thrush, *Candida* species) or viral inclusions of the cytoplasm or nucleus. Acute infectious esophagitis can also be discovered upon autopsy and possibly deemed contributory to the cause of death.

Three separate cases of acute infectious esophagitis are presented; one solely of fungal etiology, one of fungal etiology with embedded maggots, and one of bacterial (*S. ventriculi*) etiology—all discovered at autopsy at the Medical University of South Carolina in Charleston, SC. All three cases were forensic autopsy cases, authorized by local coroner jurisdiction. Two of the three decedents were male, with an age-range of 59–75 years old. All three decedents had a medical history significant for Hypertensive Arteriosclerotic Cardiovascular Disease (HASCVD), and all three were incidentally found unresponsive. Manner of death was natural in all three cases with the cause of death differing and only one having acute esophagitis included in the immediate cause. A literature review is performed to correlate the case findings with previously described entities, as well as discuss the etiology, the clinical, and the pathological presentations of acute infectious esophagitis.

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### Forensic Pathology, Infectious Esophagitis, Rare Etiology

## H77 Medullary Thyroid Carcinoma (MTC) With Diabetic Ketoacidosis: An Autopsy Case Report and Literature Review

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**Learning Overview:** After attending this presentation, attendees will better understand an uncommon case of MTC with ectopic Cushing's Syndrome (CS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping medical examiners in diagnosing MTC-related hypercortisolism by chemical analysis and tumor histology and by sharing an experience of an unusual immunostaining pattern.

MTC is a rare neuroendocrine tumor of parafollicular C cells. The majority of MTCs occur sporadically, but about 30% of patients are associated with Multiple Endocrine Neoplasia type 2 (MEN2) syndrome or familial MTC.<sup>1</sup> Generally, MTCs have no sign of clinical manifestation, but infrequently they develop symptoms of hypercortisolism by secretion of Adrenocorticotropic Hormone (ACTH) or Corticotropin-Releasing Hormone (CRH). Although ectopic CS is found only in 0.7% of MTC, it can lead to fatal consequence if left untreated.<sup>2</sup> There have been over 50 cases reported of MTC-related CS, and very few autopsy cases exist in the literature.

A 22-year-old woman was found dead in her residence. Her father stated that she lost weight in recent months in spite of no chronic illness and could not eat well after wearing a brace on her teeth in July. At autopsy, the body was significantly thin in appearance with a body mass index of 7.8kg/m<sup>2</sup>. The surface of the thyroid gland was unremarkable; however, the cut section revealed an ill-defined whitish lesion involving almost the entire right lobe. Other major internal organs showed no abnormalities. Microscopic examination revealed infiltrative MTC with insular growth pattern separated by thin fibrovascular cores. Immunostaining for calcitonin and chromogranin showed positive reaction in a few cells.

Clinicochemical analysis of vitreous humor showed a highly elevated glucose level at 674mg/dL. The tests for blood ketone bodies revealed a high  $\beta$ -hydroxybutyrate level of 1,304mg/L and a high acetone level of 574mg/L, confirming the diagnosis of diabetic ketoacidosis. The result of additional immunohistochemical study for ACTH was negative. The patient had no family history of MTC and germline genetic testing for RET mutation was not performed. There was no evidence of other medical disorders that could have caused hypercortisolism. Considering all the information, it was concluded that death was due to diabetic ketoacidosis, which was a complication of MTC-related CS.

There are several studies dealing with alteration in immunostainability of MTC in the course of disease progression. In one study, initially MTC showed high-intensity calcitonin staining with a homogenous pattern; however, the metastatic tumor tissue obtained at autopsy displayed diffusely negative staining for calcitonin.<sup>3</sup> This study presumed these different patterns of immunostaining was reflecting the presence of a poorly differentiated, biologically aggressive population of cells. This theory can also explain the poor immunoreactivity on calcitonin in this case. With regard to ACTH immunostaining, only a few cases are reported positive for ACTH, CRH, or Proopiomelanocortin (POMC) despite the evidence of ectopic hormonal secretion. Negative ACTH immunoreactivity has been suggested to reflect reduced cellular storage due to a high secretion rate, or inefficient translation and processing of POMC messenger RNA (mRNA).<sup>4</sup>

It is of importance for both forensic pathologists and clinicians to recognize that hypercortisolism can be the presenting symptom of MTC. Immunohistochemical detection of calcitonin and hormonal markers are helpful in determining the diagnosis of MTC and the reason for associated complications, but there are still things to consider in terms of immunoreactivity on postmortem tumor tissue.

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### Medullary Thyroid Carcinoma, Ectopic Cushing's Syndrome, Immunostaining

## H78 A Fatal Case of *Vibrio Vulnificus* Wound Infection

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**Learning Overview:** The goal of this presentation is to educate attendees about *Vibrio vulnificus* and to present the autopsy findings in a case of fatal *Vibrio vulnificus* wound infection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of an uncommon but potentially fatal bacterial infection.

**Introduction:** *Vibrio vulnificus* is a Gram negative, motile rod that is part of the normal microbiome of salt water environments. People can become infected with *Vibrio vulnificus* after ingesting shellfish or being exposed to salt water environments.<sup>1</sup> This organism has the highest fatality rate of any foodborne pathogen and is responsible for the majority of seafood-related deaths in the United States.<sup>2</sup> A case of lower extremity necrotizing fasciitis and bacterial sepsis due to *Vibrio vulnificus* diagnosed at autopsy is presented.

**Case Presentation:** The decedent was a 59-year-old male with no known medical history. He was known to drink alcohol, smoke cigarettes, and had used crack cocaine in the past. He was found prone and unresponsive on his bedroom floor by his roommate. Emergency services responded to the scene and pronounced him dead. There was no evidence of trauma. Per family, he was seen in an emergency room two days prior to his death for right ankle pain.

At autopsy, the right lower extremity had purple-red discoloration of the skin with numerous areas of blistering, skin slippage, ulcerations, and degloving of the epidermis of the sole of the right foot. Histologically, sections of the skin and the subcutaneous tissue at the right ankle showed superficial dermal edema filled with numerous rod-shaped bacterial organisms. These organisms proliferated into the dermis and subcutaneous adipose tissue. There was prominent acute and chronic perivascular inflammation and areas of necrosis in the deep soft tissue. Blood and spleen cultures came back positive for *Vibrio vulnificus*.

He was also noted to have left concentric ventricular hypertrophy and a remote myocardial infarct. His liver weighed 2,300 grams and was remarkable for steatosis and fibrosis histologically which was consistent with chronic ethanol use. The lungs had emphysematous changes grossly and microscopically, which were consistent with chronic tobacco use. Lung cultures were positive for *Staphylococcus aureus* and *Klebsiella* species. Postmortem toxicology was positive for diphenhydramine (0.26mg/L).

The cause of death was bacterial sepsis due to lower extremity necrotizing fasciitis due to *Vibrio vulnificus* infection. Chronic ethanol use with hepatic fibrosis and steatosis was a contributory cause of death. The manner of death was accident (sustained unknown injury of the right ankle with probable exposure to coastal water).

**Discussion:** While septic infections from *Vibrio vulnificus* after ingesting undercooked or raw shellfish are more common, wound infections should not be overlooked. When open wounds are exposed to water harboring *Vibrio vulnificus*, infection may occur. These infections most commonly occur while swimming, fishing, or preparing seafood. Wound infections may progress to necrotizing fasciitis and can be fatal. Infection with *Vibrio vulnificus* is more likely to occur in individuals with chronic liver disease, diabetes mellitus, and hemochromatosis. These infections are also more likely to occur in men and older individuals.<sup>3</sup>

Wound infections from *Vibrio vulnificus* are uncommon but potentially fatal. Epidemiologic data from the Centers for Disease Control have shown an increased rate of wound infections from *Vibrio vulnificus* in the United States in the past two decades.<sup>3</sup> Forensic pathologists should be cognizant of this microorganism and its connection to salt water environments and shellfish.

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### *Vibrio Vulnificus*, Wound Infection, Forensic Pathology

## H79 Sudden Death Due to Leukostasis in a Subject With Untreated Chronic Lymphocytic Leukemia (CLL)

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**Learning Overview:** After attending this presentation, attendees will have a better knowledge of the pathophysiology of death due to leukostasis in subjects with a hematologic malignancy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the possible mechanisms of sudden death due to leukostasis in subjects with CLL in the absence of preceding symptoms.

CLL is a chronic lymphoproliferative disorder characterized by monoclonal mature B cell proliferation. It comprises approximately 25% of all leukemias in the United States, with a slight preference for the male sex. CLL is usually asymptomatic and the incidental finding of lymphocytosis or lymphadenopathy typically leads to the diagnosis. Typical symptoms such as fever, weight loss, night sweats, and fatigue are present in 5%–10% of cases.

Hyperleukocytosis (white blood cell count  $>100 \times 10^9/L$ ) is present in a large proportion of patients at initial presentation, but symptomatic hyperleukocytosis (leukostasis; usually  $>400 \times 10^9/L$ ) is an extremely uncommon presentation. Clinical manifestations are secondary to the decreased tissue perfusion due to the intravascular accumulation of large aggregates of leukemic cells. These aggregates can be observed in virtually every organ, but the symptomatology is usually related to Central Nervous System (CNS) and pulmonary involvement.

The case of a 70-year-old White male with a medical history of dementia, bipolar disorder, hypertension, and obesity who was found unresponsive in his bed by his wife is reported. He did not have any symptoms before death.

Autopsy examination showed an obese male (BMI = 36.6kg/m<sup>2</sup>) with evidence of severe atherosclerotic-hypertensive cardiovascular disease, consisting of heart enlargement (570 grams) and critical (up to 90%) narrowing of the major coronary arteries. Massive enlargement of the spleen (2,100 grams) and numerous firm nodules within the spleen parenchyma were present. Diffuse lymphadenopathy and nodules in the left kidney and liver were also seen. Histology and immunohistochemistry were consistent with diffuse extranodal CLL/Small Lymphocytic Lymphoma (SLL). In addition, pulmonary, brain, cardiac, and renal small vessels were filled with leukemic cells, a finding highly suggestive of leukostasis. The leukostasis could have further reduced the blood supply to the heart muscle, which was already compromised by coronary artery disease, and to brain and lung, leading to fatal complications.

Two main theories have been proposed to explain the pathophysiology of leukostasis in leukemia: these are not mutually exclusive, and both eventually result in organ damage. The first theory centers on the idea that with a higher-than-the-standard-degree of viscosity, stasis can occur in the microvasculature due to adhesive interactions between leukemic blasts and their inherently rigid cell membrane compared to Red Blood Cells (RBCs). The second theory involves local tissue hypoxemia due to higher metabolic demand of the neoplastic cells coupled with the release of damaging cytokines.

Although extremely rare, leukostasis is a life-threatening complication in patients with CLL. Pathologists should be aware of this complication when investigating sudden deaths if autopsy evidence consistent with leukemia is observed.

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### Sudden Death, Leukostasis, Chronic Lymphocytic Leukemia

## H80 Drowning or Something Else? The Uncommon Birt-Hogg-Dubé Syndrome

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**Learning Overview:** After attending this presentation, attendees will have learned about an unusual syndrome that must be taken into consideration in cases of diving-related deaths, in particular in non-professional divers, and the diagnostic tools necessary for diagnosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relating the importance of focusing on the need to perform radiological examinations in the event of suspected drowning deaths and, in particular, to focus on a rare, but probably underdiagnosed, pathology.

The case report of a 31-year-old man found dead in the sea of Trieste, Italy, is presented. The suspected cause of death was a diving-related death due to the finding on the body of a diving mask and underwater fins. Before autopsy, due to advanced putrefactive changes, a postmortem Computed Tomography (CT) scan was performed. The results included: absence of air-fluid levels in the sinuses, the sphenoid area, and the mastoid cells; presence of right lung collapse with discrete compression and deviation from the midline of the mediastinum; presence of numerous cystic formations in both lungs. During the autopsy, leaking of multiple air bubbles from the right hemithorax was detected using the conventional pneumothorax test. The right lung appeared significantly reduced in volume with displacement of the mediastinum to the left. The left lung appeared hypo-expanded; there was absence of fluid in the oropharyngeal cavities, in the trachea, and in the large bronchi.

The conclusion of the coroner investigation was the exclusion of a death due to mechanical asphyxiation from drowning. Given the radiological and autopsy finding, the cause of death was a spontaneous pneumothorax in a bullous pulmonary disease during a free dive, likely due to the higher pressure underwater. Considering the radiological characteristics of both lungs, a Birt-Hogg-Dubé Syndrome (BHDS) was hypothesized. This syndrome is a rare autosomal dominant monogenic inherited disorder caused by mutation of the folliculin gene (FLCN) site on the chromosome 17p11.2. It is associated with skin manifestations such as fibrofolliculomas that appear in the third or fourth decade mainly in the upper part of the body, an increased risk of renal cell carcinomas, and the presence of numerous lung cysts, in particular in the basal zones of both lungs. These are associated with a high risk of numerous events of spontaneous pneumothorax starting from the third decade. To prove this hypothesis and more importantly offer a clinical evaluation and genetic counselling to the relatives, it would be necessary to carry out genetic investigations; however, given the high number of mutations of the FLCN gene and that the deceased was a German tourist, no further investigation was carried out.

### Autopsy, Postmortem CT, Birt-Hogg-Dubé Syndrome

## H81 An Autopsy of a Young Female With Abdominal Pain

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**Learning Overview:** After attending this presentation, attendees will appreciate the importance of autopsy as a tool to provide pathologic findings to clinicians and the importance of considering multiple differential diagnoses and risk factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of postmortem autopsy in correlating pathologic findings with clinical impressions for both pathologists and clinicians, especially when a discrepant or unexpected diagnosis is revealed by an autopsy.

Despite advances in diagnoses and managements of Peptic Ulcer Disease (PUD), Pulmonary Thromboembolism (PE), and their complications, PUD and PE are still responsible for a substantial amount of morbidity and mortality. The differential diagnoses of acute abdominal pain in young females are complex and extensive, including gastrointestinal, gynecological, or obstetric pathologies. Many of the pathologies are surgically or medically treatable, if diagnosed promptly.

In this presentation, a case of a 29-year-old female with abdominal pain is reported. A few months prior to her death, she complained of abdominal pain and heavy vaginal bleeding and was subsequently diagnosed with adenomyosis. She underwent hysterectomy about one month prior to her death. About three weeks prior to her death, she started to complain of abdominal pain again, but did not seek medical attention as it was believed that pain was to be expected after the surgery. Days prior to her death, her pain worsened and she developed shortness of breath with a visibly distended abdomen. She was brought to a local hospital emergency department when she became dyspneic and unresponsive. She was later pronounced deceased shortly after arrival at the hospital. Her other medical history included previous PE, obesity, hypertension, seasonal asthma, and history of heavy smoking. She had no known history of illicit drug abuse. Her medication list included Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and oxycodone. An autopsy examination revealed acute peritonitis, 2,500cc of turbid purulent ascites, and diffuse serositis on most of the serosal surfaces of the intestines and the liver. There was a perforated gastric ulcer in the gastric antrum just proximal to the pyloric sphincter. No histologic evidence of neoplasm was noted of the ulcer. The peritonitis was mostly acute, with focal fibroblastic proliferation. There was a moderate amount of thromboemboli of various diameters in the pulmonary arteries, with microscopic features consistent with antemortem blood clots that caused pulmonary thromboembolism. Mild subdural hemorrhages were also found. Toxicology reported a therapeutic level of oxycodone. The cause of death was determined to be acute peritonitis due to perforated gastric ulcer, and PE; the manner of death was natural. In review of medical records, multiple risk factors for PE were identified, including recent surgery, obesity, and a history of PE. Opioid use, recent gynecologic surgery, and lack of follow-up medical evaluation were possible confounding factors that masked pain symptoms and made prompt diagnosis of PUD or PE more challenging.

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### PE, PUD, Risk Factors

## H82 A Case of Hamartoma of Mature Cardiac Myocytes (HMCM) Correctly Diagnosed at Autopsy

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**Learning Overview:** The goal of this presentation is to illustrate the clinicopathologic features of HMCM and its differentiation from other primary cardiac tumors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of a rare primary cardiac tumor that may be detected or correctly diagnosed for the first time at autopsy.

**Case:** The decedent was a 35-year-old male with a history of cardiac fibroma clinically diagnosed in childhood and drug abuse who had an unwitnessed arrest at home with controlled dangerous substances found at the scene. He saw a cardiologist 16 months prior to death at which time he was asymptomatic; electrocardiogram showed normal sinus rhythm, left atrial enlargement, intraventricular conduction delay, and lateral T wave inversion; echocardiogram showed normal right ventricular size and function, normal left ventricular function, and a 7.1 x 5.5cm well-encapsulated echodensity on the left ventricular inferolateral-lateral wall. Autopsy and toxicology testing revealed the cause of death was fentanyl intoxication. Grossly, the decedent's heart had a 6 x 5 x 5cm, poorly-circumscribed, firm, white-gray mass involving the anterior, lateral, and posterior walls of the left ventricle. Microscopically, the mass consisted of mature hypertrophic cardiomyocytes with extensive fibromuscular disarray, areas of fibrosis and adipose tissue, and thick-walled arteries. Therefore, the mass that had been clinically diagnosed as a cardiac fibroma was actually a HMCM.

**Discussion:** HMCM is a rare benign primary cardiac tumor first described in 1988.<sup>1</sup> Between then and August 2019, there were 26 cases reported.<sup>2-4</sup> HMCM occurs more frequently in males (ratio 1.6:1) and affects all age groups (mean 32 years, range 6 months to 76 years).<sup>2</sup> HMCM most frequently occurs as a single mass in the left ventricle but may be multiple and involve the interventricular septum, right ventricle, and right atrium. Similar to other hamartomas, HMCM grows slowly and does not invade the surrounding tissue. Patients may be asymptomatic or have non-specific Electrocardiogram (ECG) findings, arrhythmia, palpitations, chest pain, dyspnea, syncope, or sudden death. In 46% of cases, the tumor was found incidentally at autopsy or during cardiac evaluation for other reasons.<sup>2</sup> HMCM is difficult to distinguish from other cardiac tumors such as fibroma and rhabdomyoma via imaging, and definitive diagnosis requires pathologic evaluation.<sup>2</sup> Grossly, HMCM is a discrete to ill-defined, white-tan, firm mass.<sup>2,4</sup> Microscopically, HMCM consists of disarrayed hypertrophic cardiomyocytes interposed with fibrous tissue, adipose tissue, and thick-walled arteries or dilated venules.<sup>1,4</sup> HMCM does not show significant inflammation, calcification, or vacuolization. In contrast, cardiac fibromas are well-circumscribed, white-tan, firm masses composed of bland fibroblasts and collagen, are often calcified, and are poorly vascularized.<sup>5</sup> Meanwhile, cardiac rhabdomyomas are well-circumscribed, white-gray, firm masses composed of immature cardiomyocytes with diffuse glycogen vacuolization.<sup>6</sup> In conclusion, HMCM is a rare benign primary cardiac tumor that may go undetected or be clinically misdiagnosed until autopsy, at which point routine histologic evaluation can provide a definitive diagnosis.

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### Hamartoma of Mature Cardiac Myocyte, Cardiac Hamartoma, Benign Cardiac Tumor

## H83 The Sudden Death of a Firefighter: A Look at On-Duty Coronary Heart Disease Fatalities

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**Learning Overview:** After attending this presentation, attendees will better understand the interaction of personal and work-related factors in precipitating on-duty coronary heart disease fatalities in firefighters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the prevalence of sudden cardiac death in the United States Fire Service and of the importance of adhering to the Firefighter Autopsy Protocol established by the United States Fire Administration.

Firefighting has been described as one of the nation's most hazardous occupations, as firefighters are subjected to many uncommon workplace stressors.<sup>1</sup> According to the National Fire Protection Association (NFPA), 4,593 firefighters have lost their lives while on duty in the United States since 1977.<sup>2</sup> People outside of fire service mistakenly assume that the majority of on-duty deaths result from burns or smoke inhalation; however, in the United States, the leading cause of line-of-duty fatality is sudden cardiac death, with cardiovascular disease accounting for approximately 45% of all firefighter duty deaths.<sup>1-6</sup> In contrast, such events account for 22% of deaths among police officers on duty, 11% of deaths among on-duty emergency medical services workers, and 15% of all deaths that occur on the job.<sup>2</sup>

Various explanations for the increased mortality from cardiovascular events among firefighters have been proposed, but it is ultimately thought to be from a combination of personal and work-related factors.<sup>3,5</sup> Personal factors associated with the development of heart disease are well known and include age, gender, family history, smoking history, and lack of exercise.<sup>5</sup> Not as widely known, however, are the ways in which specific firefighting duties can lead to, and even trigger, coronary events.<sup>3,5</sup> Therefore, whether these deaths are truly precipitated by the job and, if so, from which duties, remains an important area of concern.<sup>3</sup> Determining the cause of death of firefighters via autopsy has significant implications in providing improved understanding of fire scene hazards and the effectiveness of firefighting equipment.<sup>1</sup> It is for this reason that the Firefighter Autopsy Protocol was established by the United States Fire Administration.<sup>1</sup> It is important for those involved in death investigation to become familiar with these protocols so as to better assist in improving the health and safety of future firefighters.

A 44-year-old female firefighter with a past medical history of familial hypercholesterolemia, hyperlipidemia, major depressive disorder, and laparoscopic total hysterectomy went into cardiac arrest in front of co-workers while on the job. Earlier that morning, she completed annual physical ability testing and subsequently responded to two emergency calls. Upon returning to the fire station, she complained of severe chest pain and collapsed. She was transported to a local hospital, but resuscitation efforts were unsuccessful. In the emergency room, she was diagnosed with non-ST elevation myocardial ischemia.

Autopsy findings were significant for a complete blockage of the left anterior descending coronary artery in its proximal segment. The remainder of the autopsy was essentially unremarkable. Toxicological analysis revealed the presence of her psychiatric medications and an antihistamine, all of which showed concentrations within therapeutic limits. Vitreous electrolyte studies were non-contributory. Her cause of death was certified as "Occlusive coronary artery disease due to atherosclerotic cardiovascular disease." The manner of death was certified as natural.

Given the team dynamics associated with being a firefighter, on-duty adverse health events can jeopardize job performance and the safety of co-workers.<sup>6</sup> These events also have the potential of compromising public safety and impose significant economic burden on fire departments and local communities.<sup>6</sup> Because of this, it is important to understand the interaction of potential firefighting-related triggers of sudden cardiac death with underlying heart disease prevalent among firefighters.<sup>6</sup> With over one million firefighters currently working in the United States, this knowledge is important for reducing the incidence of cardiovascular events, which is of major importance to fire departments, the medical community, and society as a whole.<sup>6</sup>

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### Firefighters, On-Duty Death, Firefighter Autopsy Protocol



## H84 Subdural Hematoma Due to Central Nervous System (CNS) Histoplasmosis in the Setting of Undiagnosed Human Immunodeficiency Virus (HIV) Infection

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**Learning Overview:** After attending this presentation, attendees will be familiar with a rare presentation of CNS histoplasmosis in HIV-positive individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community because acute subdural hematoma is almost always due to head trauma and this case will expand the differential diagnosis of the causes of that lesion. Additionally, the differential diagnosis and considerations important to the diagnosis of disseminated histoplasmosis at autopsy will be discussed from the point of view of the forensic pathologist.

**Case Report:** The decedent, a 29-year-old man from Mexico was in the United States visiting family, complained of cough, intermittent fever, nausea, and vomiting for approximately one week before being found down and unresponsive in his bedroom. He did not have a history of head trauma, headache, neck stiffness, altered mental status, or photophobia. He was transported to the hospital where an acute subdural hematoma was detected.

On admission, he was febrile and tachycardic and had a faint maculopapular rash. His lab results were remarkable for a normal white blood cell count with lymphopenia and normal platelets. His International Normalized Ratio (INR) was 1.4. He had a mild elevation of his hepatic transaminases. Chest radiography showed bilateral central interstitial and airspace opacities. On neurologic examination, the pupils were fixed and dilated, and there was extensor posturing with noxious stimulus. A Computed Tomography (CT) scan of the head showed a hyperdense right hemispheric subdural hematoma, 1 mm thick, with right-to-left midline shift of up to 15mm causing effacement of the ambient cistern and entrapment of the left lateral ventricle. Following neurosurgical consultation, comfort care was initiated, and the decedent died two days later.

At autopsy, there was no sign of a head impact. The edematous brain was compressed by a 120mL right subdural hematoma without signs of organization. It was associated with uncal and subfalcine herniation and Duret hemorrhage of the pons. The heart, kidneys, liver, and endocrine glands were grossly normal. The lungs were edematous and consolidated. Microscopic analysis revealed small yeast morphologically consistent with *Histoplasma capsulatum* in all tissue samples, including the dura. Identification was confirmed by molecular identification of *Histoplasma* DNA in formalin-fixed paraffin-embedded tissue. Postmortem serum was positive for HIV-1. The cause of death was acute subdural hematoma due to CNS involvement by disseminated Histoplasmosis due to HIV/AIDS.

**Discussion:** Disseminated histoplasmosis, an AIDS-defining illness, is associated with CNS involvement in 10%–50% of patients. Clinical diagnosis is difficult because of the multiplicity of signs and symptoms associated with the disease. The clinical presentation may mimic stroke, migraine, meningitis, vasculitis, and neurosarcoidosis. A case of disseminated histoplasmosis with CNS involvement presenting with sudden obtundation as a result of an acute spontaneous subdural hematoma is presented. This has been rarely described.<sup>1</sup> The etiology of the subdural hematoma in this case is uncertain. Considerations include coagulopathy due to bone marrow involvement by the infection with resultant cytopenias or liver infiltration with associated coagulation factor deficits. Alternatively, histoplasmosis may cause a necrotizing vasculitis resulting in cerebral infarcts. None of these explanations was clearly the mechanism of the acute hematoma in this case. Clinicians and pathologists should be aware of this rare presentation of an opportunistic fungal infection in HIV-infected individuals as a possible differential diagnosis for otherwise unexplained acute subdural hematoma.

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### Histoplasmosis, Subdural Hematoma, HIV

## H85 The Importance of the Autopsy in Unknown Infectious Diseases

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**Learning Overview:** After attending this presentation, attendees will be able to better comprehend the importance of the postmortem examination in the management of unknown diseases. The current pandemic COVID-19 infection has re-marked this fundamental concept both for forensic and public health management.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing scientific data on the importance of the autopsy in the management of unknown diseases. Particularly, this presentation re-marks the pivotal role of the postmortem investigation in the identification of the main characteristics of the unknown infection, such as COVID-19.

The current outbreak of COVID-19 infection, which started in Wuhan, Hubei province, China, in December 2019 is an ongoing challenge.

The role of autopsy still remains unchanged, despite the decline in autopsy rates. During the past few decades, several factors such as time constraints, attitudes (of clinicians, pathologists, families, administrators), and costs (professional, overheads) have negatively influenced the autopsy rate leading to the lack of substantial information.<sup>1</sup> In 1996, Schwartz and Herman highlighted the importance of the autopsy, particularly in emerging and re-emerging infectious diseases.<sup>2</sup> For example, performing the histological and immunohistochemical investigations on the lung specimens collected during the autopsies of eight subjects who died of Severe Acute Respiratory Syndrome (SARS), the pathology of diffuse alveolar damage was described, providing the basis for therapeutic strategies.<sup>3</sup> Moreover, during the recent outbreak of the Middle East Respiratory Syndrome (MERS), the value of this investigation tool was emphasized. Particularly, analyzing the data reported by Ng et al., the autopsy provided unprecedented, clinically-relevant insights about how this unknown infection had progressed, challenging previously accepted ideas about MERS.<sup>4,6</sup>

In this presentation, a literature review has been performed with the aim of highlighting the importance of autopsy in COVID-19 infection. Analyzing the histological and immunohistochemical findings, COVID-19 infection generates similar lung injuries to those observed in SARS-CoV and MERS-CoV.<sup>7,8</sup> As previously described, COVID-19 infection can cause different forms of Acute Respiratory Distress Syndrome (ARDS) due to diffuse alveolar damage and diffuse endothelial damage.<sup>9,10</sup> Moreover, the postmortem investigation in COVID-19 patients can be considered fundamental in order to establish the pro-thrombotic scenario. Indeed, considering the thrombotic aspect of 21 COVID-19 subjects from the Menter et al. study, the cause of death was attributed to widespread alveolar damage in the exudative phase with capillary congestion accompanied by microthrombi despite anticoagulant therapy.<sup>11</sup>

COVID-19 infection was initially classified as exclusively interstitial pneumonia with varying degrees of severity. Subsequently, thanks to the postmortem investigation and investigating vascular biomarkers, it has been shown that it can also be considered a vascular disease, changing the therapy adopted. In this way, the importance of the autopsy has been confirmed to define the exact cause of death, thus providing useful clinical and epidemiologic information, particularly regarding emerging or unknown infectious diseases.

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### Autopsy, Infectious Diseases, COVID-19

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## **H86 Acquired Tracheoesophageal Fistula (TEF) Secondary to Tuberculosis in a Human Immunodeficiency Virus (HIV) Patient: A Case Report With Review**

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**WITHDRAWN**

## H87 Autism Spectrum Disorder (ASD) and Sudden Cardiac Death: Is There a Link? A Case Report and a Literature Review

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**Learning Overview:** The goal of this presentation is to investigate an association between ASD, the use of antipsychotic drugs, and sudden cardiac death.

**Impact on the Forensic Science Community:** The ASD and the use of antipsychotic drugs may be the cause or a risk factor of sudden cardiac death. This presentation will impact the forensic science community by focusing on the association between ASD, the use of antipsychotic drugs, and the sudden cardiac death in young people in order to obtain a reliable postmortem diagnosis.

ASD is a neurodevelopmental disorder, including qualitative impairments in social interactions and communication with repetitive and stereotyped patterns of behavior.<sup>1</sup> In 2010, there were an estimated 52 million cases of ASDs around the world affecting 60–70 children per 10,000.<sup>2</sup> Males are affected about four times more frequently than females.<sup>2</sup> Compared with mortality statistics from the general population or general population controls, the risk of premature mortality has been estimated to be two-fold to ten-fold higher in the ASD population.<sup>3</sup> Sudden unexplained death has been noted as a cause of death in individuals with autism, above all among those who also have the comorbid condition of epilepsy or intellectual disability.<sup>2</sup> There is an increased mortality in ASD due to diseases of nervous, circulatory, respiratory, and digestive systems, as well as congenital malformations.<sup>3</sup> A systematic review demonstrated that people with ASD have an increased risk of sudden cardiac death; Bilder et al. identified cardiac, respiratory, and epileptic events as the most common causes of death.<sup>3</sup> The use of antipsychotic drugs has been related to sudden death, too. The most severe consequences of treatment are arrhythmias and sudden cardiac death due to blockade of cardiac channels.

**Case Report:** A 30-year-old male with ASD, oligophrenia, and hyperkinetic syndrome was in a psychiatric clinic. He had undergone antipsychotic therapy for 15 years and would self-harm, including fingernail abrasions and blunt injuries. One morning, he was found dead on the floor of his bathroom by a nurse. A crime scene investigation was performed; the hospital room was tidy and only a bloodstain on the floor was detected. The postmortem examination was unremarkable. A complete autopsy was performed two days after death. Cervical and thoracic organs were dissected with Virchow's technique (one by one). The macroscopic examination of the brain was unremarkable. After fixation, the heart was examined. There was thickening of the left ventricular wall with asymmetrical hypertrophy with a maximal increase in the interventricular septum. A histological examination of all organs using Hematoxylin-Eosin (H&E) was conducted. H&E samples of the heart showed hypercontraction of cardiomyocytes; extremely short sarcomeres; the breakdown of the whole contractile apparatus; eosinophilic cross-bands and a granular aspect of the whole cell, and finally contraction band necrosis. According to macroscopic and microscopic autopsy findings, death was caused by sudden cardiac death.

Shavelle et al. showed that 22 of 200 deaths in ASD patients were due to cardiac disease.<sup>4</sup> Another study attributed the death of patients with ASD in 6 of 25 cases. Moreover, the use of antipsychotics drugs has been recognized as an independent risk factor for sudden cardiac death. In conclusion, using the medical records, crime scene investigation, macroscopic and microscopic heart findings, previous literature, and the use of antipsychotic drugs, this death was attributed to a sudden cardiac death.

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### Autism Spectrum Disorder (ASD), Antipsychotic Drugs, Sudden Cardiac Death

## H88 The Role of Histopathology Deaths Due to Pulmonary Thromboembolism in Forensic Cases

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**Learning Overview:** After attending this presentation, attendees will understand the role of histopathology in the postmortem evaluation of thromboembolism.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need of correct histopathological investigations in order to clarify the time of embolism and any malpractice profiles.

Pulmonary embolism represents a cardiovascular emergency that can obstruct the pulmonary blood vessels and induce severe acute decompensation of the right ventricle even if potentially reversible. The diagnosis is difficult to formulate and may be overlooked due to an atypical clinical presentation. Unfortunately, the gold standard in its identification is the autopsy that allows us to investigate thromboembolic pathology as a cause of sudden death. A fundamental role beyond the autopsy is undoubtedly the histopathology that allows us to distinguish an embolus from a postmortem clot. Specifically, the importance of immunohistochemistry in the differential diagnosis is examined. After the macroscopic examination, the histological evaluation is necessary to observe the chronological changes of a thrombus.

In this study, a retrospective analysis of the autopsy, clinical and circumstantial data of 1,718 deaths was carried out, selecting among these only the cases in which the autopsy confirmed and/or highlighted a Thromboembolism (TE). Overall, 64 cases were selected to which two cases of judicial autopsies were added, for a total of 66 cases, 29 of which were males with an average age of 71 years and 37 were females with an average age of 77 years. Of the 66 cases of established Pulmonary Thromboembolism (PTE), only the cases in which the autopsy confirmed PTE as a cause of death and cases in which the PTE was found in the trunk of the pulmonary artery or in its branches were collected. From this selection, 22 cases were analyzed. For each case, a histological evaluation was performed with hematoxylin-eosin staining. More samples were taken at the proximal, intermediate, and distal levels, along the vascular tract of adhesion of the thrombotic material, evaluating the possible presence of different periods of formation of the thrombus. Fifty-six samples were obtained. In the immunohistochemical investigation, the markers used were: CD 15, CD 68, TNF, CD31, CD 34, P-selectin,  $\alpha$  actin, collagen IV, and fibronectin. The results found that anti-CD15 and anti-CD 68 were positive in all tested samples; anti-TNF was positive in 70% of samples; anti-CD 31 was positive in 75% of samples; anti-CD34 was positive in 75% of samples; the anti-P-selectin antibody was negative in tested samples; the anti- $\alpha$ -actin was positive in 60% of samples; and the anti-collagen IV was positive in all the samples. This immunohistochemical analysis allowed, in all cases, to know the time of TE.

This study highlighted the importance of histological analysis of emboli with the examination of the wall of the blood vessel that contains it, thus avoiding removing it from the arterial branch where it is found. It is important to consider the time elapsed between death and autopsy and the time in which the body remains outside the cold room as the timing influences decomposition and the dating of thromboembolic material. In conclusion, the role of the autopsy with histological and immunohistological testing describes new methods for dating pulmonary thromboemboli.

### Forensic Sciences, Histopathology, Thromboembolism

## H89 The Influence of Body Mass Index (BMI) in Autopsy Procedures: An Experimental Study in Forensic Cases

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**Learning Overview:** After attending this presentation, attendees will understand the role of BMI in autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the influence of BMI on the postmortem schedule, on the techniques used, on the technical aspect in qualitative terms, and on the supervision of the operators.

BMI is an easy measurement to diagnose obesity. It is an anthropometric measure. BMI is based on the calculation of a person's weight in kilograms divided by the square of their height in meters. It is not affected by other variables; for this reason, it is currently the weight reference index for the quantitative classification of weight excesses.<sup>1</sup> Its importance is already known in the clinical setting, as a high BMI value is a risk factor for cardiovascular diseases. The influence of BMI in the autopsy timing and in the supervision of the operators has not been evaluated.

Twenty-five decedents who died of natural causes were selected. BMI was calculated before each autopsy dissection. The bi-mastoid cut was used for the head; for the opening of the chest and abdomen the Y-shaped incision was used. The total duration of each autopsy was timed. All autopsies were carried out in the morning, between 7.00 a.m. and noon. The average time to complete an autopsy varies significantly depending on the case to be analyzed; for natural deaths, the average is about four hours. For standardization, the operators remained unchanged at every autopsy. For all the autopsies, a standard protocol was used with removal and dissection of all the organs. Only the heart was retained and preserved in formaldehyde.

In order to evaluate the level of supervision of the first and second operators, the Mackworth clock test was chosen. The test was administered at the beginning and end of each autopsy. The duration of the test was five minutes. The clock test was designed by Mackworth in 1948 at the request of the Royal Air Force (RAF).<sup>2</sup> The test consists of looking at a round clock on which you can see a black hand turning. The subject must press a button each time the hand makes two clicks in a second rather than one. The operator receives a positive feedback (green light) when he/she recognizes the double click and presses the button correctly. In contrast, the operator receives a negative feedback (red light) when he/she cannot recognize the double click of the second hand. In five minutes, the hand performs ten times two clicks in a second.

Results were processed in specific tables. The results showed that the duration of the autopsy was directly proportional to an increase in BMI. Time to complete an autopsy was about three hours with a BMI of 25. In cases with a BMI of 40, the duration was four hours. The analysis of the results on the vigilance test confirmed that the duration of an autopsy correlated negatively with the state of vigilance of the operators. Indeed, with an increasing time to complete an autopsy, there was a decrease in the number of critical stimuli recognized from the clock test. When the autopsy lasted three hours, the operator (the first) on average, made three mistakes. When the autopsy lasted four hours, the operator (the first) on average, made five errors. There was also a negative influence of increasing BMI on the quality of the organ evisceration, in particular with the liver, pancreas, adrenals, uterus, and prostate.

In conclusion, an increasing BMI is directly related to an increase in autopsy duration. Reduction in vigilance is also correlated with the duration of the autopsy. Because a longer autopsy could compromise the quality of the same, the following recommendations are suggested with bodies having a BMI over 35: (1) increase the number of operators to divide the activities to be performed; (2) keep the body at a lower temperature (one to two degrees); and (3) use abdominal decompression techniques by removing the bowel prior to opening the chest and eviscerating the remaining abdominal organs.

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### Forensic Sciences, BMI (Body Mass Index), Mackworth Clock Test

## H90 Sudden Cardiac Death Due to Vasculitis: Case Reports and a Review of the Literature

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**Learning Overview:** After attending this presentation, attendees will understand that vasculitides are systemic diseases responsible for sudden cardiac death. Moreover, the postmortem findings could help in providing validated clinical diagnostic criteria and promoting a timely diagnosis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the usefulness of an accurate postmortem macroscopic and microscopic analysis of sudden cardiac death due to vasculitides for improving the epidemiological and pathophysiological data on these diseases.

Vasculitides are a group of disorders characterized by inflammation of blood vessel walls; they can cause various organ disorders depending on the size of the affected blood vessels. Although the classification algorithm for systemic vasculitides is periodically updated, there are currently no validated clinical diagnostic criteria. These diseases can often involve the aorta and coronary arteries and cause Sudden Cardiac Death (SCD) in subjects without any prior symptoms. Here, two cases of SCD due to vasculitis, showing different macroscopic and microscopic findings, are reported along with a brief literature review.

**Case 1:** A 43-year-old woman with sudden onset of new symptoms, including malaise, dyspnea, and retrosternal pain, underwent ergonomic testing. During the warm-up phase (about 50 seconds from the start), she suddenly lost consciousness and fell to the ground; ventricular fibrillation was observed on the monitor at the time. The woman died despite cardiopulmonary resuscitation. An autopsy was performed. Gross examination of the aorta showed intimal thickening with a fibrous, pearly-white appearance similar to fibrous plaques and a wrinkled appearance on the surface of the intimal aortic sinus (“tree barking” effect). At routine histology, there was severe narrowing of the left coronary ostium showing severe intimal thickening and inflammation; the left anterior descending artery showed obstructive intimal proliferation and thrombosis; some areas of coagulative necrosis were present in the myocardial tissue. Toxicological analysis was negative.

**Case 2:** A 34-year-old woman was found dead in her bed. Relatives stated that the young woman never suffered from any illnesses or diseases. The autopsy revealed a cardiac tamponade associated with an aortic dissecting aneurysm rupture. Histological analysis of the aorta showed fibrosis and diffuse leucocytes infiltration of the vessel's dissection edges and fibrinoid necrosis of the tunica media. Severe intimal hyperplasia of coronaries was observed. Myocardial tissue showed interstitial fibrosis and hypertrophy of myofibers. Toxicological analysis was negative.

In both cases, the deaths occurred unexpectedly and suddenly and were related to the aortic and/or coronaries damage mediated by a large vessel vasculitis with findings suggesting Takayasu arteritis.

The literature highlighted that the postmortem diagnosis of this disease can be performed by macroscopic and microscopic investigations. Postmortem Computed Tomography (CT) as well as the clinical presentation can also offer useful information. Autopsy studies suggest that large vessel vasculitides are more common than reported in epidemiological studies, suggesting the importance of an accurate postmortem diagnosis.

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### Sudden Cardiac Death, Vasculitis, Forensic Pathology

## H91 Death From Peritonitis Due to Multiple Intestinal Perforations: Approaching a Case of Advanced Tuberculosis (TB)

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**Learning Overview:** After attending this presentation, attendees will better appreciate the pathologic features associated with disseminated TB infection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a case in which death was attributed to intestinal complications of TB that are infrequent or underestimated in developed regions of the world.

Tuberculosis is typically a chronic infectious disease caused by the bacillus *Mycobacterium tuberculosis*. The transmission of the infection generally occurs through air diffusion of droplets. The natural course of the infection is extremely variable and influenced by the general condition of the subject. Cases with latent TB can be asymptomatic, while in other individuals, active TB with symptomatic disease can develop. The lungs are usually the primary site of tubercular infection, but other organs can also be affected due to the spread of *Mycobacterium* in the blood.

This case report concerns a 41-year-old man living in poor socio-economic conditions who was found dead at home. Clinical information about the subject revealed a previous diagnosis of tuberculosis. For this reason, a clinical autopsy was requested in order to determine the cause of death and establish a possible link with the infection.

External examination of the body showed almost complete disappearance/atrophy of muscles. Internal examination revealed left pleural adhesions with bilateral effusions of yellow-green fluid. Both lungs were heavy and affected by multiple cavities of different sizes, containing caseous material. Opening the abdominal cavity revealed a considerable amount of brownish fluid; inspection of the bowel revealed several granulomas with multiple perforations of the small intestine. Following the investigation, the clinical history, and the autopsy findings, a diagnosis of stercoraceous peritonitis due to tubercular intestinal perforation was made. Death due to intestinal perforation complicating intestinal tuberculosis is uncommon. The finding of multiple intestinal perforations poses questions regarding the time of onset as well as the survivability of this involvement.

In conclusion, although the knowledge of tuberculosis infections is extensive, the autopsy approach to such cases still requires vigilance given the rarity of disseminated tuberculosis in developed countries and the repercussions of this disease going untreated.

### Tuberculosis, Intestinal Perforation, Autopsy Investigation



## H92 The Dentist's Nightmare: A Case of Massive Left Ventricular Infarction After Dental Implantation

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**Learning Overview:** After attending this presentation, attendees will appreciate some of the sometimes fatal complications of dental implant surgery.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by discussing a case of sudden death in a 54-year-old man who died about two hours after a dental implant surgery that required the administration of articaine.

Sudden death is a rapid, unexpected, and unpredictable event that occurs without the possibility of clinical evaluation and in apparently healthy subjects in normal activities or in patients already diagnosed in an apparently benign phase of disease. Sudden cardiac and non-cardiac deaths fall into this category. Sudden cardiac death is a natural and sudden event in which death from cardiac causes occurs, with the sudden loss of consciousness within one hour of the onset of symptoms or in cases of unassisted death from cardiac causes in subjects alive in the previous 24 hours.

This report presents the case of a 54-year-old man found in poor health on the driver's side of his regularly parked car. Two hours earlier, he had undergone a dental implant operation that required the administration of an anesthetic of the articaine type. Given the absence of complications, the dentist discharged him and prescribed antibiotic therapy and naproxen for pain. Shortly thereafter, the man phoned his wife and reported chills and cold sweats and subsequently began to feel sick. Timely resuscitation maneuvers and intervention by health workers were unsuccessful.

On external examination, only the signs of dental intervention were visible on element 3.6 and a puncture mark on the mucosa of the oral vestibule in the vicinity of the surgical site. At the autopsy, the only finding was that of pulmonary edema. The heart was fixed in 10% buffered formalin consistent with the protocols for sudden death. Similarly, organ and fluid samples were taken for histological and toxicological investigations. Blood samples were also taken from the femoral vein for the determination of tryptase in order to exclude anaphylaxis.

The fixed heart was subjected to Postmortem Cardiac Magnetic Resonance (PMCMR) that revealed the presence of areas of altered intensity throughout the left ventricle compatible with early ischemia.

Examination of the coronary arteries on the fixed heart showed the presence of non-critical stenosis (maximum 50%) of the anterior descending and circumflex arteries. In the section of the myocardium, there were discolored areas suggesting ischemia; therefore, targeted sampling was also carried out on the basis of the imaging findings. Histology allowed confirmation of the diagnostic suspicion of early ischemia by demonstrating the presence of areas of myocytes necrosis, as well as non-specific alterations such as myofiber break-up and wavy myocardial fibers.

The toxicological tests carried out on blood and urine samples made it possible to detect only the presence of articaine in the urine sample, compatible with the therapeutic use of articaine.

At the end of the investigations, the cause of death was an extensive myocardial infarction due to a coronary spasm probably induced by the therapeutic doses of articaine.

In conclusion, the autopsy investigation, the histological review, the PMCMR, and the toxicological investigations are indispensable tools in postmortem investigations. This approach to postmortem diagnosis is essential to investigate the cause of death, especially in cases of forensic interest where it is necessary to discern between natural events and cases possibly attributable to inadequate health care.

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### Sudden Cardiac Death, Anesthetic Drug, Autopsy Investigation

## H93 A Rare Case of Sudden Cardiac Death Associated With Isolated Congenital Coronary Artery Anomalies (CAA): Autopic and Histopathological Results

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**Learning Overview:** The goal of this presentation is to demonstrate the importance of an early diagnosis of anomalies of origin of the coronary arteries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating a case of sudden death due to CAA in a young girl and the analysis of the autopic and histopathological results.

CAAs are the second cause of Acute Coronary Syndrome (ACS) under 35 years of age, after hypertrophic cardiomyopathy.<sup>1-8</sup> ACS usually occurs during or shortly after strenuous exercise. In most cases, people with CAA are asymptomatic. The anomaly is recognized only during the autopsy. The prevalence of CAA in the population is between 1%–2%.<sup>9</sup> CAAs are divided into: anomalies of origin, course anomalies, and termination anomalies.<sup>10</sup>

A case of a young student who complained of dyspnea after physical exertion for several years is presented. The girl was found dead in bed. At autopsy, examination of the coronary artery circulation showed that the Right Coronary Artery (RCA) ostium was born at the union between the right and left cusp. The right ostium also had a higher origin, specifically, the ostium was 0.4cm higher than the left ostium. The anomalous ostium had a “flute beak” shape. The first section of the RCA passed between the pulmonary trunk wall and the aorta. The inter-aorto-pulmonary extension was 0.4cm. The coronary artery circulation was left dominant. The left coronary artery showed no anomalies. The atria and ventricles were normal. The mitral valve showed myxoid degeneration with a “parachute” appearance. The other valves were normal. The histology of the heart showed wavy myocardial fibers; interstitial and perivascular sclerosis; dilation of small intramyocardial and subepicardial vessels.

The cause of death was an arrhythmia secondary to an anomaly of the right coronary artery origin. The abnormal origin of the right coronary artery from the left Valsalva sinus is documented in 0.03%–0.17% of patients who undergo traditional angiography. This anomaly is associated with ACS because the first section of the coronary artery has an inter-arterial course. In fact, the first section is compressed during systolic expansion causing ischemia in the downstream areas. This compression typically occurs during physical exercise. On the other hand, if the course is retro-aortic, pre-pulmonary, or septal, there would be no risk of ACS because the walls are not compressed. When a young patient experiences symptoms during exercise, evaluating the coronary circulation is recommended. In young people, Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) angiography as an initial screening is recommended. In this way, candidates can be selected for life-saving surgery.

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### Forensic Sciences, Anomalous Origin of Right Coronary, Sudden Cardiac Death (SCD)

## H94 Sudden Death Due to Takayasu Arteritis: A Case Report

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**Learning Overview:** After attending this presentation, attendees will understand the clinical features, differential diagnosis, and classification of Takayasu Arteritis (TAK). Attendees will also learn about pathophysiology and about the gross and histopathological autopsy findings of TAK.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to find out the cause of death in sudden cardiac death. A better approach to sudden cardiac death in a tropical region will be discussed, specifically the rare autopsy findings in TAK.

TAK is a chronic vasculitis that mainly affects the aorta, its major branches, and the pulmonary arteries. Since the description of the first case by Mikito Takayasu in 1908, several aspects of this rare disease, including the epidemiology, diagnosis, and the appropriate clinical assessment have been defined. TAK, also known as pulseless disease, occlusive thromboaropathy, and Martorell syndrome is a chronic inflammatory arteritis affecting large vessels. Vessel inflammation leads to wall thickening, fibrosis, stenosis, and thrombus formation. Symptoms presented as end organ ischemia in TAK. More acute inflammation can destroy the arterial media and lead to aneurysm formation. Early reports suggested that the disease was confined to females from Eastern Asia, but it has now been recognized worldwide in both sexes, although disease manifestations vary between populations. The female-to-male ratio appears to decline from Eastern Asia toward the West. TAK is rare, but most commonly seen in Japan, South East Asia, India, and Mexico. In 1990, it was included in the list of intractable diseases maintained by the Japanese government and to date, 5,000 patients have been registered. A study of North American patients by Hall et al. found the incidence to be 2.6/million/year.<sup>1</sup>

In this case, a 40-year-old woman had chest pain and fell unconscious; she was taken to hospital where she was declared dead. There was no history of hypertension, diabetes, ischemic heart disease, or other significant medical history. On autopsy, she was of average build and there were no external injuries. On dissection of the heart, a papillary growth was found near the left coronary artery ostia, partially blocking the ostia. The papillary growth was submitted for histopathological examination. This examination revealed the papillary growth was a mural thrombus with features of TAK. The cause of death was given as sudden death due to TAK. There is no sudden cardiac death registry in India; in the 21<sup>st</sup> century such a registry in India would be valuable.

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### Sudden Death, Takayasu Arteritis, Papillary

## H95 Subcapsular Hepatic Hematoma in a Pregnant Woman With No Prenatal Care: A Case Report

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**Learning Overview:** The goal of this presentation is to document a rare but frequently fatal complication in pregnant women: subcapsular hepatic hematoma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the important role of a postmortem examination in patient care by: (1) providing autopsy findings and cause of death; (2) raising awareness of the existence of this rare, but often fatal, complication when evaluating etiologies of abdominal pain in pregnant women; and (3) taking proper measures to prevent and treat the complications.

A 35-year-old female, G2P1 (two pregnancies, one birth), who was pregnant at 33-34 weeks gestational age, presented to a local hospital emergency department with sudden onset of severe abdominal pain. The patient was alert upon arrival. She had no prenatal care and denied using drugs. An ultrasound study detected no fetal heart tones or movement. The patient was in distress and presented with no vaginal bleeding. A chest Computed Tomography (CT) with contrast showed "partially imaged suspected hemoperitoneum." Uterine rupture or ectopic pregnancy were considered. An intraosseous line was placed. The patient continued to decompensate, with ultrasound demonstrating free fluid in the abdominal cavity. The patient then experienced sudden cardiac arrest, and resuscitation and emergency cesarean section were started. A stillborn fetus was delivered while resuscitation efforts continued. Upon initial incision into the abdomen, a significant amount of blood was noted, estimated at 1500cc. Fluid and blood were transfused and other aggressive resuscitative measures were attempted with no obvious improvement. The death was ultimately pronounced during surgery.

At autopsy, the liver was covered by a large subcapsular hematoma involving a majority of the diaphragmatic surface and half of the visceral surface. The hematoma measured 24 x 15 x 2cm in dimension. There was a large rupture of the hepatic capsule on the anterior diaphragmatic surface. The hemorrhage extended to the retroperitoneal area, including areas adjacent to the pancreas. No evidence of adenoma or hemangioma of the liver was identified. There were numerous packing cloths and regular cloths present in the abdominal cavity. Microscopically, liver tissue adjacent to the capsule showed intrasinusoidal and periportal infiltrates of neutrophils and lymphocytes with hepatocyte necrosis. Residual hemorrhages were seen in the liver parenchyma. The stillborn fetus was of 34 weeks gestational age and was normally developed with no congenital malformations. No other abnormalities were noted of the decedent or the placenta from the autopsy. The cause of death was determined to be massive hemoperitoneum due to ruptured subcapsular hepatic hematoma. There was no autopsy evidence of severe coagulopathy, liver neoplasm, or liver parenchymal injury. There was no reported history of trauma or recent surgery.

Subcapsular hepatic hematoma is often associated in pregnancy with Hemolysis, Elevated Liver enzymes, Low Platelet count (HELLP), preeclampsia, or eclampsia. Other possible etiologies are trauma, coagulopathy, hepatic neoplasm, or idiopathic etiology. In this case, some of the potential etiologies, such as HELLP, preeclampsia, or eclampsia, could not be properly evaluated due to the lack of prenatal care. Clinical symptoms and signs of rupture in the beginning are often non-specific and mimic signs of gynecological emergencies. These are all confounding factors that make the early diagnosis and treatment of subcapsular hematoma challenging.

### Liver Hematoma, Pregnancy, Autopsy



## H96 A Case of Fatal Hemorrhage During Pregnancy

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**Learning Overview:** After attending this presentation, attendees will have learned about the placenta accreta spectrum, including pathologic findings and risk factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of placenta accreta spectrum to aid in its consideration as a differential diagnosis in cases of sudden death in pregnancy.

The case of a 31-year-old G2P1 (two pregnancies, one birth) female with a history of substance abuse, prior caesarian section, and preterm delivery who was brought to the hospital at 38 weeks pregnant due to possible onset of labor will be presented. During examination at the hospital, she appeared agitated, and further evaluation and examination was difficult due to her status. She stated to health care providers that she had recently used methamphetamine. Initial assessment found no fetal heart tones, which was confirmed by follow-up ultrasound. Her agitation reportedly increased and while awaiting admission to the hospital, she became unresponsive. Attempted resuscitative efforts were not successful.

Autopsy revealed at well-developed gravid female with evidence of resuscitative efforts. There was no significant external trauma. Internal examination revealed approximately three liters of liquid blood and clot were present within her peritoneal cavity. Further examination showed a gravid uterus, with a 20cm span of abnormal dark dusky discoloration over the anterior surface. This abnormality was determined to be the maternal surface of the placenta. A 0.5cm hemorrhagic defect was present within the maternal surface of the placenta and uterus. There was no evidence of infection or lesions within the placenta. A third-trimester fetus without anomaly or injury was within the intact amniotic sac. Meconium was identified within the amniotic fluid. Microscopic examination confirmed the absence of uterine wall over an otherwise unremarkable placental tissue, consistent with placenta percreta.

Toxicology results included the presence of methamphetamine, amphetamine, buprenorphine, and norbuprenorphine in the decedent's blood. The cause of death was signed out as complications of placenta percreta, with acute methamphetamine intoxication as a contributory factor. The manner of death was accident.

Placenta percreta is part of the placenta accreta spectrum, in which the placenta abnormally invades into and through the myometrium of the uterine wall. The placental villi invade and adhere to the myometrium, which increases the risk for postpartum hemorrhage and rupture. The main pathologic classifications within the placenta accreta spectrum include placenta accreta, increta, and percreta, in which the villi invade superficially, deeper into the myometrium, or through the myometrium, respectively. While relatively rare, the rates of placenta accreta spectrum have been increasing in the past 40 years, some believed in part to an increasing caesarian section rate. Antenatal diagnosis of the placenta accreta spectrum is important due to the increased risks to morbidity and mortality of mother and fetus and for optimal management of the condition at the time of delivery.

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### Placenta, Pregnancy, Hemorrhage

## H97 A Sudden Pediatric Death: A Case of Asymptomatic Muscular Dystrophy

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**Learning Overview:** After attending this presentation, attendees will better understand: (1) the signs and symptoms of Duchenne Muscular Dystrophy (DMD), (2) its fatal complications, and (3) possible histopathological changes associated with DMD.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of sudden deaths in young children who have not yet lost ambulation due to respiratory complications of DMD.

DMD is an X-linked disorder that affects 1 in 3,300 males born in the United States.<sup>1</sup> A mutation of the dystrophin gene on Xp21 causes membrane instability and loss of calcium homeostasis in skeletal and cardiac muscle fibers. This results in muscle necrosis and subsequent regeneration, with fibrosis and fatty infiltrate replacing muscle when regenerative capacity is reached.<sup>1</sup> Bilateral progressive muscle weakness begins around the age of 4 years; the proximal muscles of the lower limbs are most severely affected. Common signs are “Gowers maneuver,” where the child uses their arms to push to a standing position, tip-toe walking, and calf “pseudohypertrophy” from fatty infiltration. Most patients will be wheelchair-bound by age 12, and life expectancy is about 26 years.<sup>2</sup> Fatal complications of DMD include cardiomyopathy and respiratory failure.

Described here is the case of a 3-year-old Arab male with no significant past medical history who was in his usual state of health. He was at home with his mother when he was reported to have complained of stomach pain while eating, turned blue, and became unresponsive. Emergency medical services responded and initiated cardiopulmonary resuscitation. He was intubated at the hospital but was unable to be resuscitated. Of note, his mother had reported that the child had red urine on the day of his death.

At autopsy, external examination showed a normally developed, normally nourished boy with no evidence of traumatic injury. There was no significant calf hypertrophy. Internally, there was bilateral pulmonary edema (combined weight: 293.1 grams, normal: 166 grams). Postmortem toxicology was negative. A postmortem Creatine Kinase-MB isoenzyme (CK-MB) was unable to be obtained due to hemolysis, and no urine was present at the time of autopsy. Microscopic examination including Movat staining of the heart showed no evidence of fibrosis or cardiomyopathy. The lungs showed scattered foci of atelectasis and there was mild cerebral edema with gliotic changes.

Histopathological examination of the diaphragm, rectus femoris muscle, and psoas muscle revealed myopathic changes consistent with DMD. This included groups of regenerating muscle fibers, small degenerating fibers, and areas of fibrosis and fatty infiltration. A recent diagnostic workup showed that the decedent had tested positive for dystrophin gene mutation. A maternal uncle had died of unknown cause at age 14, and the mother was determined to be a carrier. A creatinine kinase prior to death was 26,040U/L. The cause of death was certified as complications of muscular dystrophy and the manner of death was natural. Myopathy of the diaphragm can cause diaphragmatic paralysis, leading to respiratory failure and sudden death.

A review of the literature reveals that inactivity following loss of ambulation leads to atrophy of intercostal muscles, scoliosis, and chest wall deformity, which contribute to a decline in respiratory function and earlier demise.<sup>3</sup> Unexpected deaths in children with DMD have occurred due to aspiration pneumonia, acute respiratory distress following exercise, cardio-respiratory arrest following minor trauma with no fractures, and multi-organ failure.<sup>4</sup> Less information is available about the mechanism behind these sudden deaths in younger children/teens with DMD and more research is needed to understand the disease progression in different cases.

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### Duchenne Muscular Dystrophy, Respiratory Failure, Pediatric Death

## H98 Sudden Death After Bounce House Activities: A Late Complication of Congenital Diaphragmatic Hernia (CDH)

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**Learning Overview:** The goal of this presentation is to highlight the variability in presentation of a CDH, including age of onset and precipitating factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that late onset CDH is an important diagnosis to consider in children with acute gastrointestinal or respiratory symptoms that are not otherwise explained.

**Hypothesis:** It is hypothesized that the following patient died from late complications of a congenital diaphragmatic hernia that was provoked by increased intra-abdominal pressure associated with bouncing in a bounce house, an etiology that was not found elsewhere despite a thorough literature search.

A 3-year-old White female with no significant past medical history was found unresponsive and apneic after several hours of vomiting. The patient had reportedly not felt well since jumping in a bounce house at a festival earlier in the day. After one hour of attempted resuscitation by emergency medical services and hospital staff, the patient was pronounced deceased.

On autopsy, there was a three-centimeter opening in the left posterolateral hemidiaphragm with the spleen, stomach, and portions of small and large bowel displaced into the left chest cavity, resulting in compression of both lungs and the heart to the right side of the chest. The right lung weighed 295 grams and the left lung weighed 73 grams. Histologic examination revealed atelectasis in the left lung and congestion in the right lung, spleen, stomach, and bowel.

The cause of death in this patient was ultimately ruled to be respiratory compromise and associated gastrointestinal complications due to diaphragmatic herniation of abdominal contents into the chest cavity. It is believed that this sequence of events was triggered by jumping in a bounce house earlier in the day, which created a pressure gradient significant enough to allow the abdominal organs to herniate into the chest cavity.

CDH is a condition characterized by failure of the diaphragm to form completely *in utero*, resulting in an opening through which abdominal organs can invade.<sup>1</sup> Most commonly occurring posterolaterally, called a Bochdalek hernia, CDH is typically diagnosed prenatally or at birth, though in some cases it can be asymptomatic until later in life.<sup>2,3</sup> Theories for why this may occur include occlusion of the opening by the liver or spleen, by a sac associated with the abdominal contents, or by the rim of the defect itself. Late onset of symptoms has been reported at times of increased intra-abdominal pressure, such as coughing, burping, or flying.<sup>4-7</sup>

CDH can be difficult to diagnose in children because of the rarity of presentation beyond infancy. It should be considered in patients who present with acute onset of gastrointestinal or respiratory symptoms without a known etiology or appearing out of proportion to possible etiology.<sup>8</sup>

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### Congenital Diaphragmatic Hernia, Intra-Abdominal Pressure, Bounce House

## H99 Intraosseous Puncture-Induced Bone Fractures Mimicking Child Abuse: A Case Report

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**Learning Overview:** After attending this presentation, attendees will be aware of the possibility of Intraosseous (IO) puncture-induced fractures mimicking child abuse and be familiar with its associated radiological and autopsy findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting appropriate case investigation and improving wound interpretation in IO puncture-induced bone fractures, especially in decedents with congenital or acquired conditions that affect bone formation, thus avoiding misinterpreting these wounds as child abuse-associated injuries.

**Introduction:** IO puncture is frequently used to rapidly establish vascular access in critically ill patients when venous routes are not available. In generally healthy adults, the procedure typically only causes minor local puncture injury. However, in some cases, more severe injuries may occur, especially in patients who have congenital or acquired conditions that affect bone formation. In pediatric decedents, the skeletal fractures induced by medical interventions such as IO puncture may occasionally become so severe that they may radiographically simulate child abuse and cause diagnostic difficulty.

**Methods:** A case of a teenage decedent whose antemortem IO punctures is reported; it caused extensive bilateral long bone fractures that raised initial concerns for child abuse.

**Results:** A 15-year-old male with a history of Duchenne muscular dystrophy underwent autopsy. He had only relatively vague complaints prior to death. Antemortem radiographs showed bilateral humeral neck fractures with slipped epiphyses of indeterminate age, which raised concerns for child abuse related injuries. The bones appeared demineralized and gracile. External examination demonstrated symmetrical atrophy and external rotation of bilateral lower extremities as well as foot deformity, compatible with the decedent's reported Duchenne muscular dystrophy history. Multiple minor abrasions, contusions, areas of erythema, and needle punctures were present on the limbs. Bilateral IO lines were present in the humeral heads, with abundant associated hemorrhage and underlying fractures, which corresponded to the antemortem radiological findings. Autopsy examination also demonstrated subtle bilateral lower lung lobe consolidation and left ventricular cardiac discoloration. Microscopic examination revealed myocardial changes consistent with Duchenne muscular dystrophy and histologic evidence of lobar pneumonia. Bone marrow emboli were present in the pulmonary vasculature, which was consistent with being due to resuscitation-related fractures. The decedent's medical records were carefully reviewed. The decedent received extensive resuscitation efforts, including IO punctures, prior to death. Taking the pattern of the injury and the decedent's medical history into consideration, the case was ruled as being due to complications of Duchenne muscular dystrophy including pneumonia. The manner of death was categorized as "natural."

**Conclusions:** Medical interventions, such as IO puncture, occasionally cause more severe and extensive bone damage in people who have disorders that affect bone formation. Duchenne muscular dystrophy is a genetic disorder that causes progressive muscle weakness, which leads to frequent falls, immobility, and eventually, death. It is associated with increased bone fragility due to lack of use and side effects of prolonged glucocorticoid therapy. In the pediatric population, such injuries may raise concerns for child abuse-related injuries. Thorough autopsy examination with careful medical history review is essential for the appropriate interpretation of these injuries, so they are not misinterpreted as abusive trauma.

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### Intraosseous Puncture, Mimic, Child Abuse





## H100 Pediatric Cylindrical Battery Ingestion Causing Fatal Aorto-Esophageal and Pulmonic-Esophageal Fistulae

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**Learning Overview:** After attending this presentation, attendees will understand that both cylindrical and button batteries continue to pose a fatal ingestion risk to children. Attendees will appreciate how battery ingestion may cause delayed death secondary to fistulae formation between the esophagus and adjacent great vessels and how vascular anomalies in pediatric patients (such as those with DiGeorge Syndrome) may further complicate their clinical picture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the importance of correlating medical history, clinical imaging, autopsy, and scene investigation in establishing cause of death in fatal pediatric ingestions. This case also highlights the ongoing need for education regarding risks of battery ingestion to parents and caregivers.

Pediatric battery ingestion is a well-characterized cause of accidental injury and death. Due to their erosive properties, batteries can cause fistula formation between the esophagus and great vessels when ingested, leading to massive bleeding. The following case report demonstrates a fatal pediatric cylindrical battery ingestion in a child with DiGeorge Syndrome.

The case is that of a 4.5-year-old Hispanic male who reportedly swallowed an AAA battery at an unknown time at home. Two days prior to hospital presentation, his parents stated he was choking on something, given a drink of water, and appeared to no longer be in distress. The next day, the child had throat pain, dysphagia, fever, and vomiting and was brought to the hospital. A Computed Tomography (CT) scan was performed, showing a battery in the child's esophagus, which was subsequently removed. There was alkaline erosion noted. Following removal, he had multiple complications, including persistent nosebleeds and an influenza infection. Twenty days into his hospitalization, he developed bleeding of the oronasal cavity, went into cardiac arrest, and was unable to be resuscitated. Of note, the child had a history of DiGeorge Syndrome with associated overriding aortic arch and dysphagia, juvenile rheumatoid arthritis, and osteopenia.

Autopsy revealed two parallel transmural ulcerations of the mid-esophageal mucosa. One ulcer traversed the aortic wall creating an aorto-esophageal fistula, and the second ulcer formed an esophageal-pulmonic artery fistula. There was bilateral, geographic aspiration of blood into the lungs. There was 15 grams of clotted blood and 150cc of liquid blood in the stomach and duodenum. The kidneys were pale. The heart weighed 125 grams, and the aorta exhibited a right-sided aortic arch anomaly crossing over the right bronchus, then running posterior to the trachea and esophagus, and descending along the thoracic spine. Additionally, the left subclavian artery arose from the proximal descending portion of the thoracic aorta. Microscopic examination confirmed communicating esophageal fistulas between the aorta and pulmonic artery with surrounding granulation tissue, acute and chronic inflammation, and hemosiderin-laden macrophages.

Cause of death was esophageal fistulas of aorta and pulmonic artery due to battery ingestion. The manner of death was accident.

The literature reveals battery ingestion most frequently occurs accidentally in children and that button batteries are most commonly ingested. The poison control database cites 62 reported cases of fatal battery ingestions between 1977 and 2019, including the current case. This is the only reported case of dual aortic and pulmonic artery fistulae secondary to cylindrical battery ingestion. Combined with the patient's dysphagia, the patient having DiGeorge syndrome with a vascular malformation may have led to an increased risk of complications.

Pediatric cylindrical battery ingestion can cause delayed death in children by formation of esophageal-vascular fistulae, and while first described more than 40 years ago, it remains a preventable cause of death in children. Children at increased risk for this complication may include those with aberrant vascular anatomy and those with dysphagia. It is crucial for the medical examiner and investigators to gather all pertinent medical and scene history when an autopsy is requested in such a case.

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### Battery Ingestion, Aorto-Esophageal Fistula, DiGeorge Syndrome

## H101 A Fatal Ruptured Saccular Middle Cerebral Artery Aneurysm in a 14-Year-Old Boy: A Case Report

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**Learning Overview:** After attending this presentation, attendees will have an increased awareness of the characteristics and etiology of pediatric saccular aneurysms.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing features of a rare cause of intracranial bleeding in a pediatric patient.

Intracranial aneurysms rarely occur in patients less than 18 years of age and only represent 0.5% to 4.6% of intracranial aneurysms.<sup>1</sup> As a result, pediatric aneurysms are poorly understood compared to their adult counterparts. In adults, risk factors are associated with the development of intracranial aneurysms, such as smoking, hypertension, diabetes, high fat and cholesterol diets, obesity, and excessive chronic alcohol intake. However, these are generally not yet present in children. Trauma and infections are the biggest contributors to pediatric aneurysm formation. Additional contributors include: gene mutations interfering with the extracellular matrix; connective tissue diseases, including Marfan's syndrome; coarctation of the aorta; Ehler-Danlos syndrome; fibromuscular dysplasia; and polycystic kidney disease.<sup>2</sup> This report seeks to add to the limited literature on this subject.

This is the case of a 14-year-old male who was found unresponsive in his bed the morning after an uneventful night. He was previously well and had no familial history, history of traumas or drug use. He had recently started exercising and swimming. He was pronounced dead at the hospital emergency department that day.

Postmortem non-contrast Computed Tomography (CT) was conducted that revealed a Subarachnoid Hemorrhage (SAH), which prompted further investigation. A postmortem CT angiogram was performed that showed a ruptured berry aneurysm and an SAH, also seen on pre-angiography Magnetic Resonance Imaging (MRI). Post angiography MRI confirmed the ruptured saccular aneurysm and SAH. On autopsy examination, the aneurysm was measured at 0.8mm in the widest dimension and was located at the bifurcation of the right Middle Cerebral Artery (MCA) and the right ophthalmic artery.

Microscopic examination of the vessel dilatation confirmed a saccular aneurysm of the right MCA with disruption of the elastic lamina. There was no evidence of significant atherosclerosis or inflammatory infiltrates. Toxicology of postmortem blood was negative for ethanol. Genetic analysis for aortopathy and Ehlers-Danlos was negative.

This case is an example of a ruptured non-traumatic, non-infectious saccular aneurysm with no known predisposing factors. The literature states that roughly 30% of aneurysms in children are idiopathic.<sup>3</sup> More research is needed to determine the etiologies of these presentations in order to diagnose them before they rupture, although this is difficult due to their rarity. This case contributes to the literature by demonstrating a thorough postmortem investigation including CT angiography, MRI, autopsy examination, and ancillary testing in a pediatric ruptured saccular aneurysm.

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### Saccular Aneurysm, Subarachnoid Hemorrhage, Pediatric



## **H102 A Molecular Autopsy by Exome Sequencing in a Decedent With Suspected Genetic Cerebral Small Vessel Disease**

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**WITHDRAWN**

## HI103 Retinal Hemorrhages (RH) in a 6-Month-Old Child Related to Disseminated Intravascular Coagulation

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**Learning Overview:** After attending this presentation, attendees will understand that there are many causes of RH in children and will be able to develop a broad differential diagnosis for a child presenting with RH in addition to Abusive Head Trauma (AHT).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing physicians about the importance of considering disseminated intravascular coagulation as a potential cause of RH and urge them to remember that RH are not always indicative of AHT.

Child victims of AHT often present with a triad of extensive RH, subdural hemorrhage, and encephalopathy.<sup>1</sup> RH are seen in 50%–100% of fatal cases of AHT in young children <3 years old; typical findings include RH and vitreous hemorrhages, retinal folds, retinoschisis, and optic nerve sheath hemorrhage. However, other causes of RH must be excluded. Differential diagnoses for RH include accidental trauma, intracranial hemorrhage, and cardiopulmonary resuscitation, as well as coagulopathies, hematologic malignancies, vasculopathies, meningitis, arteriovenous malformations, and metabolic disorders.<sup>2</sup> This report presents the case of an unresponsive infant who was resuscitated and was noted, on clinical exam, to have RH, raising the concern of AHT.

A 6-month-old child was found unresponsive in an adult bed, where he had been sleeping next to his father. Following prolonged (> 2 hours) cardiopulmonary resuscitation, he was eventually resuscitated. Ophthalmologic examination revealed RH, raising the concern of possible AHT. The child died 8.5 hours after hospital admission. Except for focal subscalpular hemorrhage, there was no trauma noted at medicolegal autopsy. The brain had early hypoxic-ischemic changes and the eyes had rare RH; there was no evidence of subdural hemorrhage or optic nerve sheath hemorrhage. The cause of death was certified as sudden unexplained infant death with a contributing factor of an unsafe sleep environment. A review of hospital clinical laboratory values indicated that the infant had Disseminated Intravascular Coagulation (DIC), which likely contributed to the RH.

Children with AHT often have a triad of extensive RH with subdural hemorrhage and encephalopathy. In this case, extensive medical intervention and clinical laboratory findings consistent with disseminated intravascular coagulation likely explain the child's RH. It is recommended that physicians are aware of the importance of considering disseminated intravascular coagulation as a potential cause of RH and remember that RH are not always indicative of AHT.

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### Retinal Hemorrhages, Abusive Head Trauma, DIC

## H104 Death in the Water? Not Always Drowning!

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**Learning Overview:** After attending this presentation, attendees will understand the importance of external examination, autopsy findings, and histological and immunohistochemical studies in a singular case of a child dying as a result of acute fulminant myocarditis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the necessity of a complete autopsy and histopathological examinations to assess the real cause of death when it would seem simple.

Acute fulminant myocarditis is a disease of unclear pathophysiology and etiology, uncertain diagnosis, and variable presentation.<sup>1</sup> The World Health Organization (WHO) and the International Society and Federation of Cardiology (ISFC) define myocarditis as an acute, chronic, or fulminant inflammatory disease of the heart muscle, diagnosed by the histological, immunological, and immunohistochemical method.<sup>2</sup> A variety of etiologies, including systemic illness, drugs, and infectious agents, have been implicated; however, for a proportion of cases, the cause remains unknown.<sup>3</sup> Clinical presentation of myocarditis range from mild dyspnea and non-specific symptoms (fever, chest pain) to life-threatening ventricular arrhythmias, cardiogenic shock, and sudden death.<sup>4</sup> Children more frequently have fulminant myocarditis. The histological diagnosis of myocarditis is based on the manifestation of an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes.<sup>5</sup> Immunohistochemical techniques improve the diagnosis through characterization and quantification of myocardial inflammatory reactions.<sup>6</sup> The immunohistochemical investigations show an increased number of CD45R0+-T-lymphocytes in myocardial samples.<sup>6</sup>

**Case Report:** A 6-year-old male was found dead at sea. He was taken out of the water, but, despite cardiopulmonary resuscitation, the doctors certified the death. External examination did not show any visible injuries on the body and no plume of froth at the mouth and nostrils. In the fingers, a blueish coloration was observed; a white foamy, frothy material was also observed on the naris. A complete autopsy was performed two days after death. Cervical and thoracic organs were dissected with Gohn's technique (*en bloc*). No significant findings were observed during the autopsy, and there were no signs of froth in the trachea. The macroscopic examination of all organs was unremarkable. Histological examination of all organs using Hematoxylin-Eosin (H&E) staining was performed. Lung samples showed edema and abundant inflammation. The H&E-stained heart samples showed diffuse round cell infiltration with focal necrosis of cardiac myocytes. A marked lymphohistiocytic infiltrate was present diffusely in all chamber walls, accompanied by histiocytes and plasma cells. Immunohistochemical staining was performed on the lungs and heart, utilizing antibodies directed against helper T cells (CD4), cytotoxic T lymphocytes (CD8), and B lymphocytes (CD20). The immunohistochemical-stained lung samples showed a strong positivity for CD8 lymphocytes. The immunohistochemical-stained heart samples showed a strong positivity to CD4, CD8, and CD20, diffusely in all chamber walls.

The external examination and the macroscopic and microscopic findings contributed to an exclusion of a drowning death. The histological examination and the immunohistochemical study of the heart allowed the death to be attributed to an acute fulminant myocarditis in an apparently healthy child.

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### Fulminant Myocarditis, Immunohistochemistry, Inflammatory Cardiomyopathy

## H105 A Particular Case of Adipositas Cordis in a Girl Suffering From Lymphocytic Myocarditis and Hashimoto's Thyroiditis

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**Learning Overview:** After attending this presentation, attendees will have learned about a very unusual case of Adipositas Cordis (AC) in a young 14-year-old girl suffering from myocarditis and Hashimoto thyroiditis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing how the forensic investigations were conducted for the diagnosis of AC in this rare case.

AC is a rare and poorly understood heart disease. This is proved by the fact that there are no guidelines on the subject. AC is characterized by structurally normal myocytes and diffuse adipose cell infiltration in the myocardium of both ventricles, especially in the right. In some cases, it could be a diagnostic challenge as it can closely mimic Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). Preliminarily, there is no standard method for diagnosing AC before sudden death.<sup>1</sup> Secondly, in the scientific community, it is not known if AC is the first stage of ARVC. In fact, they could be two completely different diseases.

The primary differentiating characteristics between AC and ARVC are myocyte degeneration and interstitial fibrosis that are present in ARVC and responsible for the higher risk of Sudden Cardiac Death (SCD).<sup>2</sup> A case of AC is evidenced by prominent fatty infiltration associated with lymphocytic myocarditis and Hashimoto's thyroiditis is presented.

The young girl was apparently healthy and had a normal life, regularly playing sports. She had no symptoms. The girl was found dead at home with no apparent cause. During the autopsy, the organs were removed and fixed in formalin. Small portions of ventricular myocardium and 300ml of peripheral blood were frozen at -80° for further investigation. Formalin-fixed samples were stained with hematoxylin-eosin. Screening for drugs was carried out on blood and urine, after extraction with trichloroacetic acid and acetonitrile with immunoenzymatic method, gave negative results. The search for genetic material for the most common viral agents of myocarditis, with the Polymerase Chain Reaction (PCR) technique, on the heart and blood was also negative. Since myocarditis is associated with ARVC in some cases, the genome for known mutations for ARVC was examined with negative results.<sup>3</sup>

Histological investigation showed acute lymphocytic myocarditis on the anterior wall of the left ventricle, most likely on an autoimmune basis, and chronic lymphocytic thyroiditis. Adipose infiltration involved a large part of the right ventricle and extended for two-thirds of the wall. In some regions, the AC extended to the endocardium. There were no fibrous replacements. Myocytes did not show ARVC-typical changes.

With the results of the investigation, the SCD was attributed to AC. It is unlikely that the arrhythmic event was triggered by myocarditis, because it was too small and unique, and the central necrosis was not very extensive. Most likely in AC, the conduction of the electrical impulse is compromised by the dissociation of cardiomyocytes.

It is now known that AC is frequently associated with acute coronary syndrome. There have been a number of sudden AC deaths in recent years. The scientific community around the world must pay attention to AC, especially as affected patients have no symptoms and no related Electrocardiogram (ECG) abnormalities.<sup>4,5</sup> Unfortunately, AC can only be confirmed after autopsy; the real cause of AC remains unknown.<sup>6,7</sup> AC needs to be further explored, and the diagnostic criteria must be determined. Until the cause of AC is discovered, forensic pathologists should look for known ARVC mutations in these cases. In fact, many authors consider AC to be an early form of ARVC.

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### Forensic Sciences, Adipositas Cordis, Hashimoto's Thyroiditis

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## H106 Hand Sanitizer Ingestion Leads to Lethal Methanol Toxicity

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**Learning Overview:** The goal of this presentation is to present a case study regarding an individual who died of unintentional methanol toxicity due to hand sanitizer ingestion and to make the forensic community aware of the possibility of methanol as an unlisted ingredient in hand sanitizers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a case of a chronic alcoholic known to consume alcohol-based hand sanitizer who died of unintentional methanol toxicity.

Alcohol-based hand sanitizers are commonly used as a quick alternative to hand washing for the purpose of hand sanitation. According to the United States Food and Drug Administration (FDA), 97% of hand sanitizers contain one of three active ingredients: ethanol, isopropyl alcohol, and benzalkonium chloride. The Centers for Disease Control and Prevention (CDC) recommend using alcohol-based hand sanitizers containing greater than 60% ethanol and 70% isopropanol as active ingredients.

Recently, multiple brands of alcohol-based hand sanitizer have been found to contain methanol as an unlisted ingredient. Methanol, also known as wood alcohol, is a toxic alcohol that is widely used as a solvent. Methanol is typically found in industrial products, antifreeze, de-icing solutions, windshield wiper fluid, cleaners, and fuels. Methanol can appear in hand sanitizer as an unlisted ingredient due to contamination or manufacturing. Methanol is an unacceptable ingredient in hand sanitizers as it can be absorbed through the skin and lungs, or potentially ingested, causing methanol toxicity.

Methanol toxicity can lead to a decreased level of consciousness in a manner similar to ethanol (alcohol). In addition, if treatment is not sought early in the course of toxicity, the liver will convert methanol into formic acid. Formic acid accumulation can lead to metabolic acidosis, vision disturbances, seizures, end-organ toxicity, coma, and death. Treatment options for methanol toxicity include supportive care, fomepizole, dialysis, and folate.

This case report describes the death of a 34-year-old man with a history of heavy alcohol use. Due to an inability to obtain alcohol, he began consuming hand sanitizer with an active ingredient of ethyl alcohol (70%) for an unknown period of time. The night prior to his death, he complained of visual difficulties and was found unresponsive several hours later. Following his death, his spouse found numerous empty containers of the same brand of hand sanitizer hidden around the house. There were no other sources of methanol within the residence.

Postmortem examination revealed a well-developed man with a bicuspid aortic valve and nephrolithiasis. There were no other significant gross or microscopic abnormalities. Toxicology analysis of postmortem femoral blood detected only a significantly elevated level of methanol (370mg/dL). No other substances were present on postmortem toxicology analysis, including a screen for ethylene glycol.

The cause of death in this case was attributed to the toxic effects of methanol and the manner of death was accidental. Because the decedent was known to consume alcohol-based hand sanitizer and numerous empty bottles of the same hand sanitizer were found hidden in his house following his death, the source of the methanol was believed to be hand sanitizer. The purpose of this case report is to make the forensic community aware of the possibility of methanol as an unlisted ingredient in hand sanitizers, leading to lethal, unintentional methanol toxicity.

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### Methanol, Hand Sanitizer, Chronic Alcohol Abuse

## H107 No Laughing Matter: Deaths Associated With Chronic Nitrous Oxide (N<sub>2</sub>O) Abuse

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**Learning Overview:** The goal of this presentation is to describe mechanisms by which chronic recreational N<sub>2</sub>O use can contribute to death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the neurological and hematological sequelae of chronic N<sub>2</sub>O abuse.

N<sub>2</sub>O is an inhalant anesthetic, propellant in the food industry, and used recreationally by some inhalant abusers. A case series of three decedents with histories of chronic recreational N<sub>2</sub>O abuse, and the associated neurological and hematological sequelae associated with N<sub>2</sub>O use, is presented.

**Case 1:** The decedent was a 57-year-old male who was found deceased in bed. His medical history included daily inhalation of 20 to 50 canisters of N<sub>2</sub>O, ethanol abuse, diabetes mellitus, macrocytic anemia, and lower extremity weakness with difficulty ambulating thought to represent Guillain-Barre syndrome. Autopsy findings included cardiomegaly, moderate coronary artery atherosclerosis, and steatosis of the liver. Microscopically, the spinal cord showed demyelination, presence of macrophages, and reactive astrocytes of the posterior and lateral columns. Postmortem (femoral) blood N<sub>2</sub>O concentration was 34mcg/mL.

**Case 2:** The decedent was a 43-year-old male who was found deceased on his couch. He reportedly had been lying on the couch for several days prior to death inhaling canisters of N<sub>2</sub>O almost continuously. He had a clinical history of difficulty ambulating, which was attributed to vitamin B12 deficiency associated with chronic N<sub>2</sub>O inhalation. He was treated with vitamin B12 injections and over-the-counter supplements. Autopsy findings included consolidations of the lungs. Microscopically, the lungs had acute pneumonia, and the spinal cord showed mild demyelination, rare macrophage, and gliosis of the lateral columns. N<sub>2</sub>O was not detected in postmortem blood.

**Case 3:** The decedent was a 21-year-old female found deceased on her couch. Reportedly weeks prior to death, she had sustained a right ankle strain, limiting her mobility and causing her to utilize a wheelchair. Numerous N<sub>2</sub>O canisters were found throughout her residence. Autopsy demonstrated an obese female with pulmonary artery thromboembolism and deep venous thromboses of the lower extremities. Microscopically, the spinal cord showed demyelination, macrophages, and reactive astrocytes within the white matter tracts. N<sub>2</sub>O was not detected in postmortem blood.

N<sub>2</sub>O is a colorless and odorless non-flammable gas with various uses, including clinically as an anesthetic, as an aerosol propellant for food products, and auto racing. Inhalation of N<sub>2</sub>O, also known as "laughing gas," reportedly causes euphoria, anxiolysis, hallucination, and has a narcotic effect. Common mechanisms of inhalation include balloons filled by medical or commercial N<sub>2</sub>O tanks, and from whipped cream dispensers using N<sub>2</sub>O chargers, commonly referred to as "whippits," easily purchased over the internet. Death from acute N<sub>2</sub>O toxicity are rare, and deaths associated with N<sub>2</sub>O use are often due to a local suffocating environment, such as a plastic bag over the head.

Chronic use of N<sub>2</sub>O can have both neurologic and hematologic complications as seen with vitamin B12 (cobalamin) deficiency. In the body, cobalamin is converted to its active forms, methylcobalamin or adenosylcobalamin. N<sub>2</sub>O inactivates methylcobalamin, a cofactor for methionine synthase, resulting in decreased methionine production. This results in impaired myelination of the spinal cord causing Subacute Combined Degeneration (SCD) of the spinal cord or impaired DNA synthesis leading to megaloblastic anemia. SCD is the degeneration of white matter in the posterior and lateral columns of the spinal cord, and clinical manifestations include numbness, paresthesia, polyneuropathy, or myelopathy. Other comorbid conditions such as malnutrition associated with chronic ethanolism (as in Case 1) or pernicious anemia could be a factor in an individual developing SCD or megaloblastic anemia. Vitamin B12 supplementation may also be a reason that in Case 2, the histological changes were subtle compared with Cases 1 and 3.

Though the decedents did not die from acute N<sub>2</sub>O toxicity, all three displayed clinical or histological signs of SCD, which are associated with chronic N<sub>2</sub>O abuse. Decreased mobility associated with chronic N<sub>2</sub>O use in Cases 2 and 3 was a contributory condition for developing pneumonia and deep vein thrombosis respectively. In Case 1, the decedent also had history of megaloblastic anemia, which could be linked to N<sub>2</sub>O use as well as chronic ethanolism. In summary, chronic N<sub>2</sub>O use can have neurological as well as hematological sequelae potentially contributing to death.

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### Nitrous Oxide, Subacute Combined Degeneration, Inhalants



## H108 A Case Series of Cyanide Poisoning: A Rising Trend

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**Learning Overview:** After attending this presentation, attendees will have meaningful insights into pathologic features and toxicologic and autoptic findings in cases of cyanide poisoning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting an overview of cases of cyanide poisoning in Antalya, Turkey, after a new trend of suicides by cyanide ingestion sparked within the country in late 2019.

Cyanide is used in industrial processes for gold extraction, metal refinery, or in organic syntheses and other chemical processes.<sup>1,2</sup> Additionally, it can be detected in edibles such as apple or apricot seeds and bitter almond.<sup>3</sup> The toxicity of the substance is very well described in humans. A fatal dose of 0.15–0.3 g/person for potassium cyanide or 0.05g/person for hydrogen cyanide can rapidly inhibit the electron transportation system within cellular aerobic metabolism. Moreover, as with other toxic substances and drugs, cyanide has also been reported in literature to be used for suicidal and homicidal purposes.<sup>2</sup>

This case series consists of five cases of cyanide poisoning after emergence of a new trend of suicide by cyanide poisoning. Four of the five cases were from a murder-suicide incident. A family of four was found dead in their home and the suicide note, left by the father, revealed that he had poisoned his family and subsequently committed suicide due to his financial problems. The last is a case of a 30-year-old man found dead in his car with a note warning people about the presence of cyanide.

Histopathological and toxicological investigations were carried out for all cases following complete postmortem examinations. During the postmortem examinations, the Ghon's technique was performed with removal of the thoracic block and the rest of the internal organs according to Virchow's technique. All the cases had signs of asphyxia with cyanosis of fingernails and face, widespread petechial hemorrhages on their skin, petechial hemorrhages and hyperemia of the brain, subpleural hemorrhages, pulmonary congestion, and congestion of other internal organs. Toxicological analysis determined the presence of cyanide in fatal levels for all cases.

Cyanide is known to be an extremely toxic substance to humans, and yet in many places, as well as in Turkey, it is easily obtained; in fact, it could be found online until recently, available with just a few clicks. An incident of murder-suicide in Istanbul, two other murder-suicide incidents, a homicide, and isolated suicide cases by cyanide poisoning had occurred within a month. This trend eventually forced the government to restrict and control the sale of potassium cyanide.

In conclusion, the present case series demonstrates the need for forensic pathologists and medical examiners to be prepared for emerging trends worldwide. In this new world order of internet/electronic access to information, information can easily be disseminated and can turn an isolated incident into a new trend.

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### Cyanide, Suicide, Autopsy Investigation

## H109 Black Esophagus and Fatal Cocaine Intoxication: An Unusual Combination

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**Learning Overview:** The goal of this presentation is to highlight the case of a 68-year-old Caucasian male found dead at home with a massive hematemesis. Autopsy revealed the presence of acute esophageal necrosis and toxicological investigations identified the cause of death in acute cocaine intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing Acute Esophageal Necrosis (AON), also known as “black esophagus,” as a rare and potentially lethal complication of cocaine abuse.

**Introduction:** AON, also referred to as acute necrotizing esophagitis and “black esophagus,” is a pathological condition characterized, at endoscopic examination, by a diffuse full-circumference black appearance of the esophageal mucosa, which usually affects the distal portion of the organ and abruptly terminates at the gastroesophageal junction. It is a rare disease, with a prevalence found up to 0.2% of cases in some autopsy studies. During endoscopic investigations, its prevalence varies from 0.001% to 0.2% of cases. The etiology of AON is multifactorial, probably linked to a simultaneous presence of tissue hypoperfusion due to impaired circulation and low-flow states, alteration of mucosal protective factors and repair mechanisms in the presence of debilitation and malnutrition, and topical chemical lesions resulting from reflux of gastric acids due to conditions of esophagus-gastroparesis. The patients usually present with signs of upper gastrointestinal bleeding, such as hematemesis and melena. Other symptoms such as epigastric pain, nausea, vomiting, dysphagia, chest pain, and syncope may also be present. Multiple associated comorbidities are commonly found, the main ones being hypertension, diabetes, dyslipidemia, and widespread vascular atherosclerosis. Findings on physical examination are usually related to an underlying medical condition. Tachycardia, hypotension, shock, fever, cachexia, hypoxia, and abdominal pain are some of the signs that may be present, but none of these are specific for esophageal necrosis. Diagnosis is made by endoscopy. A biopsy is also needed to rule out infections and other causes of esophageal necrosis. Clinical management classically involves treatment of the underlying disease, intravenous fluid administration, concentrated red blood cell transfusions, absolute fasting, proton pump inhibitors, and sucralfate suspension. The evolution of the clinical picture largely depends on the severity of the underlying disease.

The possible complications of “black esophagus” include perforation of the wall with consequent mediastinal infection, narrowing or stenosis of the organ lumen, and duodenal ulcers. The mortality rate can reach up to 32% in the presence of severe comorbidities.

**Case Presentation:** A 68-year-old man at home was found in cardiac arrest with profuse hemoptysis. The man had a previous history of heart disease with pacemaker placement, diabetes mellitus treated with insulin, and renal failure undergoing dialysis treatment. The death was confirmed onsite by the medical staff. An autopsy was requested to clarify the cause of death.

**Results and Discussion:** The autopsy revealed a blackish discoloration of the esophageal mucosa at the mid-distal segment, up to the gastro-esophageal junction, with a typical “black esophagus” appearance. The presence of slight thickening of the coronary wall with diffuse atheromatous plaques was also observed, without significant stenosis of the lumens. Histological examination revealed the presence of contraction band necrosis of myocardial tissue, extensive areas of myocardioclerosis and foci of chronic inflammation of the pericardial adipose tissue. The esophageal mucosa was extensively de-epithelialized, with foci of chronic inflammation. Immunohistochemistry on the esophageal samples (CD3, CD20, BCL2, BCL6, CD10, Ki-67, CKAE1/AE3) was performed. The toxicological screening test carried out on cadaveric blood was positive for the presence of benzoylecgonine (the main inactive metabolite of cocaine), showing a quantity of 530ng/ml, and negative for other substances of abuse. The data was then confirmed by qualitative-quantitative analysis performed with gas chromatography/mass spectrometry. The cause of death was therefore attributed to cardiogenic shock following acute cocaine intake and gastrointestinal hemorrhage due to acute esophageal necrosis.

**Conclusion:** In the literature, there are very few cases of cocaine abuse associated with the onset of acute esophageal necrosis and gastrointestinal bleeding. In this regard, the case presented is of considerable interest as it supports the hypothesis that the intake of cocaine, causing vasoconstriction, can further compromise the esophageal blood circulation and trigger massive necrosis of the mucosa, especially in predisposed subjects.

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### Black Esophagus, Cocaine, Intoxication

## H110 Wischnewsky Spots and Black Esophagus in Deaths Involving Diabetic Ketoacidosis: A Case Series

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**Learning Overview:** After attending this presentation, attendees will better understand the pathogenesis of Wischnewsky spots and acute esophageal necrosis and their usefulness in determining cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing Diabetic Ketoacidosis (DKA) as an underlying etiology of Wischnewsky spots and acute esophageal necrosis. This will provide increased ability in determining cause of death.

**Introduction:** Mucosal lesions within the upper gastrointestinal tract at autopsy are relatively rare but can be important in determining an underlying cause of death. Two such lesions are Acute Esophageal Necrosis (AEN), also called “black esophagus,” and Wischnewsky spots of the gastric mucosa. This report details three cases where DKA was a primary or contributing cause of death. Black esophagus and Wischnewsky spots presented concurrently in all three cases.

**Case Reports:** Case 1 involves a 48-year-old female who collapsed on her bathroom floor. Toxic effect of methamphetamine was found to be the primary cause of death, and DKA was found to be a contributing factor. This death was complicated by hypertensive and atherosclerotic cardiovascular disease. There was no evidence of possible hypothermia.

Case 2 involves a 51-year-old jail inmate who was found unresponsive in his cell and was determined to have died of acute pyelonephritis. Similar to Case 1, the decedent had elevated levels of vitreous glucose and acetone consistent with DKA, as well as hypertensive and atherosclerotic cardiovascular disease. There was no evidence of possible hypothermia.

Case 3 involves a 51-year-old male who was determined to have died of DKA, with methamphetamine and fentanyl use being contributing factors. Uncontrolled diabetes mellitus had led to recent amputation of the great toe. The decedent was found outdoors so interpretation of the pathological findings was complicated by possible hypothermia.

**Discussion:** AEN involves mucosal necrosis of the esophagus, a finding that has been reported in few case studies that have mainly centered around alcohol abuse and diabetes mellitus but have been as far reaching as hypothermia and Steven’s Johnson syndrome. AEN is thought to be an initial ischemic process with further damage being caused by reflux of gastric contents.<sup>1</sup> Wischnewsky spots are lesions of the gastric mucosa that are classically thought to be associated with fatal hypothermia, with different studies reporting a range of 40%–91% incidence in cases where hypothermia was a contributing cause of death.<sup>2</sup> Despite being considered pathognomonic for hypothermia, Wischnewsky spots have also been seen in conjunction with DKA.<sup>3</sup> Histologically, Wischnewsky spots are characterized by autolysis of erythrocytes and capillary dilatation.<sup>2</sup> In this series, AEN and Wischnewsky spots are presented concurrently with DKA in all cases, suggesting a possible common or connected etiology, especially when considering previously reported simultaneous presentation in hypothermia. Thermogenic dysregulation and ischemia in DKA are explored as possible pathologic mechanisms.

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### Diabetic Ketoacidosis, Esophageal Necrosis, Wischnewsky Spots

## H111 Sodium Nitrite Suicide: A Case Report

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**Learning Overview:** After attending this presentation, attendees will be able to identify the gross autopsy findings associated with fatal methemoglobinemia and utilize that information in conjunction with scene investigation for the identification of cases with fatal sodium nitrite ingestion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the value of thorough scene documentation and the interplay of multiple aspects of forensic investigation in coming to the correct conclusions at the time of autopsy.

Sodium nitrite has wide uses in food preservation, pesticides, pharmaceuticals, industry, and construction. Toxic exposure to sodium nitrite is usually secondary to accidental ingestion or inhalation and results in a dose-dependent methemoglobinemia.<sup>1</sup> However, suicide through intentional ingestion of sodium nitrite is rarely described in the literature.

An autopsy case of a 25-year-old African American male with a history of depression and suicidal ideation is presented. He was found deceased in his bed at home. At autopsy, heavy foam was present within the airway and exuded from the nares. The body had a slight ashen appearance; however, no obvious abnormal coloration was noted in the visible livor, subcutaneous tissues, or organs. Pulmonary edema, bilateral pleural effusions, and mild cerebral edema were identified grossly. Blood spots on filter paper collected at the time of autopsy had a dark brown color. Initial toxicological examination was negative for common drugs of abuse and ethanol. The negative toxicology, despite autopsy findings suspicious for drug overdose, prompted additional review of the scene photographs and documentation. No drug paraphernalia was at the scene; however, the following items were noted: three bottles of sodium nitrite (anhydrous) with a mailing envelope from the Amazon.com store where it was purchased, a pack of “AAA Ormus” Advanced Artisan Alchemy pills and “Garden of Life—mykind organics” men’s vitamins. Subsequent toxicological analysis targeted for methemoglobin was performed on an autopsy blood sample; the results revealed greater than 80% methemoglobin saturation. The cause of death was attributed to fatal methemoglobinemia due to intentional sodium nitrite ingestion.

Methemoglobin is an oxidized form of hemoglobin that cannot bind oxygen. In humans, normal physiologic levels are less than 1%–2%. Levels above 70% are generally lethal, though survival has been reported with levels up to 94%.<sup>1,2</sup> The mechanism of death in sodium nitrite overdose is tissue hypoxia due to the oxidizing effects of the chemical leading to impaired oxygen binding and transport ability.<sup>1-3</sup> Patients presenting with methemoglobinemia may experience non-specific symptoms, including headache, lightheadedness, or fatigue/lethargy; these symptoms may even progress to shock and coma due to hypoxia.<sup>3</sup> At autopsy, the body may show discoloration of the *livor* ranging from blue to green to brown, and similar discoloration may be noted on blood spots.<sup>2</sup> Of note, discoloration of the blood, as seen on the blood spot on filter paper (red to chocolate brown), can suggest methemoglobinemia and the degree of discoloration is reported to correlate with severity.<sup>3</sup>

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### Sodium Nitrite, Autopsy, Forensic Pathology

## H112 Toxicological, Histological, and Immunohistochemical Analysis in a Case of Malignant Arrhythmia Due to Acute Pure Caffeine Intoxication

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**Learning Overview:** The goal of this presentation is to discuss a very rare case of suicide due to acute pure caffeine intoxication. Since caffeine acute intoxication pathological findings are non-specific, toxicological, histological, and immunohistochemical analysis are essential to prove the role of pure caffeine as cause of death.

**Impact on the Forensic Science Community:** Deaths by caffeine consumption are rarely described and are mainly related to caffeine-based medication abuse. Due to its high interindividual variability, toxic doses are extremely difficult to define. This presentation will impact the forensic science community by presenting a case that is unusual due to acute intoxication by pure caffeine consumed for suicidal purposes.

Caffeine is a naturally occurring purine-based alkaloid, mostly consumed as a psychostimulant. It can be found in different natural substances, even if its recent use has been related mainly to caffeine-based medications and energy drinks. Its effects are dose-dependent, generally causing undesirable effects with doses higher than 500mg. Only a few cases of death due to acute caffeine intoxication have been described, the majority of which have been attributable to massive consumption of caffeine-based medications, with blood concentration of 15–20ug/ml. In such cases, the cause of death is often identified as ventricular fibrillation due to catecholaminergic stimulation.

Since autopsy findings are non-specific for caffeine, toxicological analysis and the determination of caffeine concentration in the blood are essential. This work presents the case of a 39-year-old Caucasian female found dead at home. At the crime scene, a white powder was found on the victim's lips. The same powder was identified on the kitchen table, as well as in many plastic glasses in the sink, close to other empty glasses. A handwritten letter was found, confirming the suicidal intentions of the women. Samples of white powder were taken for toxicological analysis. During the autopsy, no pathology was found in the major organs, except for the lower esophagus and stomach, which were distended and their consistency was increased. The upper digestive tract contained a solid pinkish-white substance in the shape of the gastric and esophageal cavities (weight of 664g). Samples of that substance, along with blood, vitreous humor, urine, and bile samples, and major parenchymal organs were taken for toxicological analysis. Histological analysis on the brain, heart, liver, kidneys, and stomach were also performed. This revealed myocardial contraction band necrosis, suggesting fatal arrhythmia as the mechanism of death. Toxicological analysis was extremely meaningful, as it proved the presence of pure caffeine inside the gastric and esophageal cavities. Caffeine concentration in the blood was 47µg/ml, and very high levels of catecholamines and their metabolites were detected in the urine samples. The white powder sampled at the crime scene was confirmed to be pure caffeine. Immunohistochemical analysis of the myocardium demonstrated the β1-adrenoceptor involvement.

Deaths by caffeine consumption are rarely described and mainly relate to caffeine-based medication abuse. Due to its high variability among individuals, toxic doses are extremely difficult to define. Our case is unique due to an acute intoxication by pure caffeine consumed for suicidal purposes. Toxicological analyses associated with the histological signs of myocardial injury confirmed the cause of death was due to fatal arrhythmia due to pure caffeine intoxication.

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### Caffeine, Arrhythmia, Acute Intoxication

## H113 An Analysis of the Effect of Necrophagous Entomofauna on Fabric Modifications During a Summer Season in Western Australia

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**Learning Overview:** After attending this presentation, attendees will understand the effect of carrion insects on fabrics during the postmortem period. In particular, attendees will understand those variables (e.g., the nature of the fabric [natural, synthetic, blended], the fabric's elastic content [% in elastane], the type of initial damage [tear/penetration] that will most impact on how the fabric modifies during a decomposition event).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new body of information that will enhance the investigative role of clothing associated with decomposed and skeletonized remains.

Fatal stabbing incidents are the leading cause of homicides predominantly in countries with restricted access to firearms, such as Australia. During a stabbing assault, the distinctive characteristics of an implement deposit specific features, typically assessed during wound examination by a pathologist and/or during a fabric damage assessment by a forensic scientist. When the decomposition process impedes the identification and evaluation of the type and extent of a stabbing wound, fabric damage analysis on the victim's clothing may provide information about the implement or the actions that caused the injuries. However, studies have suggested that insect activity can modify the original cut (e.g., exacerbating the fraying of a fabric's cut, especially the edges of the cut). Furthermore, insect activity and the progression of decomposition such as bloating have also been reported to produce changes to clothing that in some cases may mimic indicators of sexual homicides. At present, there is a paucity of research focused on the effect of insect activity on different fabrics and the modifications they cause throughout the process of decomposition.

The aim of this study is to analyze the effects of the activity of the necrophagous entomofauna during a summer season in Western Australia on different types of fabric (natural, synthetic, blended—with different amount of elastin), type of damage (tear/penetration), and time since death/insect colonization.

For this study, 117 piglets (*Sus scrofa* L.) were used. Four different fabrics were selected based on their type (natural/synthetic) and their percentage in elastin (0%, 50%, 100%): (1) cotton 100%; (2) polyester 100%; (3) cotton-elastane 50%–50%; and (4) spandex 100%. All fabrics were woven. Of the 117 piglets, 112 were wrapped from the neck down with one layer of each fabric type and in the same weave orientation. Five piglets were not clothed and were used as controls. Twenty-four wrapped piglets were stabbed twice, consecutively and at identical anatomical positions with a Philips-head screwdriver (pointed edge) and 24 with a kitchen knife (sharp edge) by utilizing a stabbing apparatus (for consistency and to maintain a similar amount of pressure when thrusting). The fabric of 24 wrapped piglets was torn twice at the same positions as the penetrated piglets. Twenty-four wrapped piglets were left undamaged and 17 wrapped piglets were excluded from insect activity to serve as controls. Also, 112 samples of fabrics that were not wrapped on piglets were placed at the field site along with the samples. The experiment took place in a eucalypt woodland on sandy soil in southwestern Australia. The environmental conditions were also documented.

Data collection was comprehensive of piglets, fabrics sample (a total of 20 replicants every three days from the beginning of the experiment), and insect specimens (via direct collection and adhesive traps). At each sampling period, photos and videos were recorded. Analyses performed covered both taphonomic aspects (degree of piglets' decomposition), entomological (insect species and instar), and physical evidence (fabric damage via stereomicroscope and Scanning Electron Microscopy [SEM]). The statistical analysis considered the different variables (e.g., time since death, insect activity, type of fabric, type of damage) and assisted in the generation of likelihood ratios for the interpretation of damage on the fabrics.

This presentation will discuss the results of this experiment and its impact on postmortem interval assessments, as well as implications on fabric damage analysis.

### Textile Damage, Decomposition, Forensic Entomology

## H114 Inter-Individual Variation in Soil Chemistry and Microbial Ecology During Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will understand how cadaver-related factors influence chemical and microbial patterns in decomposition-impacted soil. This presentation will increase attendees' knowledge of human decomposition processes, including differential decomposition resulting from human variability, particularly as it relates to the soil environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by furthering the knowledge of cadaver-related factors (intrinsic factors), such as weight and health conditions, that influence soil chemical and biotic decomposition patterns.

Human decomposition is a dynamic process, and decomposition rate and soil chemical/ecological patterns are driven by a series of decomposer organisms, notably insects and microorganisms.<sup>1,2</sup> Prior studies demonstrate that extrinsic factors (e.g., temperature) influence decomposer activity; however, the effects of intrinsic factors (e.g., decedent body mass, disease status) on decomposer patterns have not been explored.<sup>3</sup> Decomposer activity and rate of decomposition differs between individuals experiencing the same environmental conditions, suggesting that intrinsic factors are responsible for the degree of individual variation.<sup>4</sup> Thus, the purpose of this research was to probe the effects of intrinsic factors on soil chemical and microbial decomposer responses to human decomposition.

Nineteen deceased human individuals were placed supine on the soil surface at the Anthropological Research Facility at the University of Tennessee (February 2019–March 2020) and allowed to decompose naturally. Prior to placement, each individuals' medical information (height, weight, etc.) and medical histories were recorded. Soil samples were taken at predetermined Accumulated Degree Hour (ADH) intervals until the end of active decomposition (here defined by cessation of decomposition fluid purging from the abdomen). Each soil sample was homogenized and hand-picked to remove debris (e.g., roots and insect larvae) larger than 2mm prior to analyses. To understand soil chemical patterns, soil pH and Electrical Conductivity (EC) were measured. Soil microbial activity was assessed via heterotrophic respiration and extracellular enzyme assays. Co-extracted bacterial and fungal DNA was quantified, and community composition was examined using 16S ribosomal RNA (rRNA) gene and Internal Transcribed Spacer (ITS) amplicon sequencing, respectively.<sup>5-7</sup>

Preliminary results indicate that an individual's end-of-life condition impacts soil chemistry and microbial ecology. While soil pH, EC, and heterotrophic respiration generally increased during active decomposition, change in EC over time exhibited greater reduction in individuals with respiratory illnesses than those without (Wilcoxon  $p = 0.018$ ). Principal component analysis shows that soil chemical profiles do not cluster by environmental factors, such as season or location; instead, other factor(s) are likely driving variation between individuals. Soil microbial communities changed over time and varied among individuals, based on end-of-life condition. For example, individuals with cancer had lower richness estimates and distinct community composition (Permutational Multivariate Analysis Of Variance [PERMANOVA],  $p < 0.01$ ) compared to those without cancer.

This study provides evidence that intrinsic variability between donors originating from different end-of-life conditions influences chemical and microbial patterns in decomposition-impacted soil. These results have considerable implications for the construction of microbial-based postmortem Interval (PMI) models as well as assessing error rates within those models.<sup>8</sup>

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### Differential Decomposition, Microbial Ecology, Soil Chemistry

## H115 Temperature-Dependent Postmortem Protein Degradation in Pigs

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**Learning Overview:** The goal of this presentation about temperature-dependent protein degradation and the research regarding postmortem protein decomposition for Postmortem Interval (PMI) estimation is for attendees to gain more information about an innovative method to determine the time since death—a crucial topic in forensics.

**Impact on the Forensic Science Community:** A precise determination of the time since death is of high importance in forensic routine. Further research in this field is necessary in order to expand the prevailing range of applications in forensic PMI estimation. Especially, analysis of muscle protein degradation has shown its potential to contribute to according mid- and long-term delimitations in practice. This presentation will impact the forensic science community by improving understanding on postmortem protein degradation and its dependency on temperature in particular.

A precise determination of time since death plays a major role in forensic practice. Currently available techniques for the determination of the PMI include the temperature-based method on the cooling behavior of bodies and forensic entomology, which investigates the diversity and development of necrotrophic insects on a dead body. All methods, however, are restricted to specific time periods or must be excluded under certain circumstances. Recently, it has been shown that the analysis of muscle protein degradation has the potential to contribute to the determination of the PMI. An innovative approach, based on biochemical analysis of postmortem protein degradation, has been developed that expands the range of applications in forensic mid- and long-term PMI estimates. In particular, specific time points in the degradation of certain proteins provide reasonable markers for PMI determination. Nevertheless, considerable research is yet required to increase our understanding of protein decomposition and how it is affected by individual and environmental factors in order to provide a broad application of this method. Several factors can alter protein degradation patterns, for instance individual traits (e.g., age, sex, body weight) or environmental circumstances, such as humidity, exposure to sunlight, or ambient temperature, without a doubt the most important factor.

A standardized protein degradation model was developed to biochemically analyze postmortem muscle samples at different ambient temperatures. Dismembered pig hind limbs were stored under controlled conditions at 20°C and 30°C and muscle samples (M. biceps femoris) were regularly collected at predefined time points. Sampling times were calculated according to respective Accumulated Degree Days (ADD; [°d]), which are defined as the product of time and ambient temperature (10 days at 20°C and 6.6 days at 30°C result in 200°d, respectively). This allowed a valid comparison between experiments at different ambient temperatures. All samples were further processed via Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), and selected proteins were identified by Western blotting.

Results showed precise, time-dependent degradation patterns of investigated muscle proteins in a predictable manner that were dependent on storage conditions and ambient temperature. Some proteins remained stable over the investigated time course, others showed complete degradation of the native protein band, partly accompanied by (transient) degradation products at distinct PMI phases. Though there were similarities of protein degradation patterns detectable between muscle samples stored at 20°C and 30°C, most proteins showed accelerated degradation kinetics and, in part, additional degradation events at higher environmental temperatures.

This study provides evidence that ambient temperature has a major effect on postmortem protein degradation. It remains to be investigated whether protein degradation patterns vary under low ambient temperatures. Present results, together with an additional storage experiment at ambient temperatures below room temperature, will help to develop a precise model of postmortem protein degradation depending on ambient temperature and time since death.

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### Protein Degradation, PMI, Temperature





## **H116 The Detection and Profiling of Seminal DNA From Fly Larvae, Pupae, and Puparia to Investigate Sexual Intercourse Postmortem**

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**WITHDRAWN**



## H117 The Temporal Relation of the Generation of 3-Methylbutanol and Related Compounds in Decomposing Chicken Liver

*Kelsey Springer, BS\*, New Haven, CT 06515; Robert H. Powers, PhD, University of New Haven, West Haven, CT 06516*

**Learning Overview:** The goal of this presentation is to investigate the time-dependent pattern of evolution of ethanol, 2-butanone, 3-methyl butanol, and 3-methylbutanal from a chicken liver homogenate. Attendees will learn of the potential for time-related volatile generation during the decompositional processes to be related to Postmortem Interval (PMI).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating the time-related pattern of evolution of volatile compounds during the decompositional processes to be related to PMI.

**Overview:** The hypothesis examined was that temporal differences in the appearance and concentration of volatile compounds during autolysis and decomposition could be related to the time and temperatures during which the processes occur and may therefore provide a basis for a chemical-measurement based estimation of PMI.

Homogenate of fresh chicken liver was prepared as a 1:1 ratio with water, strained, and constantly stirred for a 96-hour sampling period in 125ml erlenmeyer flasks at room temperature. Starting volume was ~ 85mL. Five 0.4ml aliquots from the autolytic chicken liver homogenate were collected at each time point for 12-hour intervals from 0–96 hours. Samples were preserved with sodium fluoride/potassium oxalate (~2.5, and 2mg/ml, respectively) and refrigerated prior to analysis. Samples were heated in an oven ten minutes prior to analysis. Headspace analysis by Gas Chromatography/Mass Spectrometry (GC/MS) was performed on the samples, and peak areas for ethanol, 2-butanone, 3-methylbutanal, and 3-methyl butanol were used to determine both the pattern of their generation and the ratios between the various species. The ratios of species generated were analyzed in accordance with the time at which they appeared in the decompositional process.

In this system, 3-methylbutanal became detectable at 12 hours, with maximal levels achieved at ~36 hours, declining thereafter. In contrast, ethanol became detectable at 36 hours, with the concentration increasing through 96 hours. 3-Methylbutanol and 2-butanone also became detectable at 48 hours with the continued increase of 3-methylbutanol while 2-butanone reached an apparent maximum at 84 hours, declining thereafter. In the experimental system utilized, generation of volatile decompositional products did not appear at similar rates, with 3-methylbutanal appearing prior to ethanol, 2-butanone, and 3-methylbutanol, and different times to maximal concentrations. While data from this artificial decompositional environment would not necessarily be applicable to any real-world situation, the results suggest the possibility that species and organ-specific volatile compound ratios may be relatable at the autolytic stage, via estimation of the temperature history of the body to the postmortem interval.

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### Decomposition, Volatiles, Postmortem Interval

## H118 The Succession of Postmortem Protein Degradation in Different Muscles in Rats and Humans

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**Learning Overview:** The goal of this presentation is to highlight the importance of investigating influencing factors on postmortem phenomena in order to apply them to determine the time of death. This presentation analyzes the dynamics of postmortem protein degradation in the context of the composition of muscle fibers and the temperature influence.

**Impact on the Forensic Science Community:** In forensic science, an accurate determination of the time of death is still a challenge and, therefore, a central topic in forensic research studies. This presentation will impact the forensic science community by detailing that although postmortem protein degradation has proven to be a valuable tool, further research is required for broad range applicability of the method for criminal investigation.

The determination of time since death is of central interest in forensic research. Therefore, new and innovative methods are under investigation in order to improve the currently available tool set. A promising new method, based on biochemical analysis of postmortem protein degradation, uses decomposition patterns of muscle proteins to determine the Postmortem Interval (PMI). To date, this technique was mainly applied on thigh muscle tissue. Sampling of this muscle may not always be possible or has to be excluded under specific circumstances, and investigation of degradation behavior of other muscles is necessary in order to provide a broader applicability of this method. In general, skeletal muscle represents the largest homogeneous body compartment and is qualified as a target tissue for this method, not only due to the high abundance and protein content but also because it is easy to sample and yet well protected by the skin. Nevertheless, it remains unclear how muscle fiber composition and/or the location of muscles within the body affect protein degradation behavior after death and whether distinct muscles show different protein decomposition patterns. Temperature, for example, has a major impact on protein degradation and decreases unevenly throughout the body after death. This, together with the proximity to the gastrointestinal system, may lead to different postmortem protein decomposition in muscles located near the body core compared to distal muscles in extremities.

To test this hypothesis, four different muscles (*M. vastus lateralis*, *M. psoas major*, *M. soleus*, and *M. gastrocnemius lateralis*) were collected from an experimental setup in rats and also from humans during autopsy in order to investigate the influence of the location within the body and decreasing temperature during cooling, as well as differential fiber-type composition. The *M. gastrocnemius lateralis* is mainly built out of fast type II muscle fibers, whereas the *M. soleus* is composed of mostly slow type I fibers, but both muscles are located in the lower leg. In contrast, the *M. psoas major* and *M. vastus lateralis* are situated in the trunk and upper leg, respectively, and have even fiber-type ratios. Samples were biochemically analyzed using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blotting. Protein degradation patterns were compared between the different muscles and species. Immunohistochemistry was performed in order to determine fiber-type composition of the investigated muscles.

Results show similar degradation patterns in animals and humans regardless of fiber-type composition. In contrast, temperature significantly affects postmortem protein degradation. Muscles in near proximity to the body core show faster degradation compared to muscles of the periphery, most likely due to different location of muscles and their respective cooling behavior after death. This study provides evidence that the location of muscle within the body has a higher impact on protein degradation than muscle fiber composition. However, there are still some factors that can additionally influence protein degradation patterns. Ambient temperature, physical conditions, or antemortem diseases may cause differences in the muscle type distribution and consequently in postmortem protein degradation. To optimize this method, further investigations and comparisons between different muscle types will be necessary.

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### Protein Degradation, Muscle, PMI

## H119 Postmortem Protein Degradation as a Tool to Estimate the Postmortem Interval (PMI): A Systematic Review

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**Learning Overview:** The goal of this presentation is to provide an overview of the research on forensic postmortem protein degradation of the past 20 years, discuss limitations and the current state of knowledge, and address future perspectives and important open research questions.

**Impact on the Forensic Science Community:** Estimation of the PMI is of critical importance in forensic routine, but often extremely difficult. Novel techniques to contribute to the current methodic spectrum are certainly required, but published research often just scratches the surface. This presentation will impact the forensic science community by highlighting the immense potential of protein degradation for PMI estimation and provides the basis for targeted future research.

**Background:** Estimation of the PMI is a critical task in forensic practice. In recent years, biochemical analysis of the postmortem breakdown of biomolecules, especially proteins, has become of significant interest in this regard. Numerous “novel methods for PMI estimation” have been proposed, addressing proteolytic processes, and according postmortem alterations in a variety of tissues. However, much of the existing work consists of basic research studies, using animal models or a small number of human cases, and/or does not take the numerous possible influencing factors, such as environmental temperature, age, or others, appropriately into account. This literature review aims to investigate the current state of knowledge and the future perspectives of postmortem protein decomposition for the use in forensic PMI estimation.

**Objectives:** A systematic review of the literature to evaluate the current research status of protein degradation-based PMI estimation is provided. Special attention is dedicated to the applicability of the proposed approaches/methods in routine forensic practice.

**Method:** A systematic review of the literature on protein degradation in tissues and organs of animals and humans was conducted. A search of the scientific databases PubMed® and Ovid for articles published between 1999 and December 2019 was performed. Additional searches were performed in Google® Scholar and the reference lists of eligible articles.

**Results:** A total of 36 studies were included. This enabled this study to consider the degradation pattern of over 130 proteins from 11 different tissues, studied with different methods, including well-established and modern approaches. Although comparison between studies is complicated by the heterogeneity of study designs, tissue types, methods, proteins, and outcome measurement, there is clear evidence for a high explanatory power of protein degradation analysis in forensic PMI analysis.

**Conclusion:** Although only few approaches have yet exceeded a basic research level, the current research status provides strong evidence in favor of the applicability of a protein degradation-based PMI estimation method in routine forensic practice. Further targeted research efforts toward specific aims (also addressing influencing factors and exclusion criteria) will be required to obtain a robust and reliable laboratory protocol and collect sufficient data to develop accurate multifactorial mathematical decomposition models.

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### PMI, Protein, Review

## H120 Multiple Sampling of Human Muscle Tissue Over Time to Eliminate Biases and Enhance Data Quality for Protein-Based Postmortem Interval (PMI) Delimitation

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge about influencing factors on muscle protein degradation, as well as on solutions to method-specific challenges, that facilitate the improvement of data acquisition and quality.

**Impact on the Forensic Science Community:** The estimation of the time since death has always been a major topic in forensic science. The future goal is to develop a practical and easily feasible method for PMI delimitation, based on molecular markers that can be used in the early and late postmortem period. This presentation will impact the forensic science community by informing attendees that further research on how internal and external conditions influence the process is needed to be able to make reliable judgements based on the method in forensic case work.

**Background:** Delimiting the PMI is a central question in routine forensic work and often still one of the most difficult challenges. The biochemical analysis of the postmortem disintegration of proteins in human muscle tissue has recently shown to be a valuable approach for this task. However, case-specific circumstances of death, and the influence of individual variables, as well as environmental conditions on protein degradation, such as Body Mass Index (BMI), age, or exposure of a body in each case, are yet to be thoroughly investigated to produce reliable results and thus allow successful application of this method to routine forensic work.

**Methods:** Samples of human muscle tissue were taken every 4–6 hours over a period of at least 24h, in ten autopsy cases, and analyzed for skeletal muscle protein degradation. The bodies were stored in a climate chamber, with controlled environmental temperature and humidity, and sampled up to nine times per case. Cases with well-known PMIs and reliable information in terms of ambient temperature were selected for this pilot project to maximize the validity of the results and allow comparisons between cases. Causes of death were diverse, initial PMIs ranged from 28 to 126 hours postmortem (hpm) and storage temperatures during the sampling period were individually chosen between 5°C to 15°C.

**Results:** By analyzing multiple samplings of human muscle tissue at different postmortem time points under controlled environmental conditions, possible biases arising from varying age, BMI, sex, and metabolic rate between individuals were eliminated and by that obtained unique outcomes. Additionally, critical challenges to overcome for future data acquisition and analysis were identified.

**Conclusion:** This study highlights the benefits of multiple tissue samplings of individuals over a period of time. In comparison to samples from single time points, this approach provides reliable, largely unbiased data on postmortem muscle protein degradation. Future research should aim to gain further knowledge about the impact of influencing factors on the process of postmortem protein degradation in human skeletal muscle tissue for this method to be confidently used in routine forensic PMI determination.

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**PMI Delimitation, Protein Degradation, Multiple Sampling**

## H121 The Identification of Unidentified Remains

*Katherine Cochrane, MD\*, Knoxville, TN 37920; Darinka Mileusnic-Polchan, MD, PhD, Knox County Regional Forensic Center, Knoxville, TN 37919*

**Learning Overview:** The goal of this presentation is to discuss the transnational and multidisciplinary collaboration required to improve the success of positive identification of unidentified remains using the National Unidentified and Missing Persons System (NamUs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging the diligent entry of unidentified remains cases into NamUs to facilitate an improved rate of successful identification.

Annually, there are estimated to be 4,400 unidentified remains cases, 1,000 of which remain unidentified after one year. Degradation of the remains by factors such as an extended postmortem interval, burning, or scattering results in the failure of traditional identification methods and necessitates the use of DNA profiling. When an identity is not immediately established, these cases may remain unidentified for extended periods, and a missing person database may be utilized to distribute the information to a larger audience and potentially find a match. These cases are a tremendous challenge, in part to the involvement of multiple agencies and specialists, as well as the need for transnational sharing of information. In response to an overwhelming need for a centralized reporting system, the National Institution of Justice created NamUs in 2007. Designed to be the nation's central database for missing and unidentified persons investigations, this web-based repository accessible to law enforcement, forensic scientists, medical examiners, and family members of missing persons, shares information nationwide, enhancing the potential for identifying remains.

Six cases of remains positively identified using DNA comparison analysis between 2016 and 2018 were examined. These included three sets of remains in an advanced stage of decomposition, two charred remains, and one consisting of scattered bone fragments. Five identifications were made by comparison with putative relatives, and one by cold hit through comparison with DNA profiles from missing persons. All cases were entered into NamUs; however, two families instead utilized a private laboratory for DNA comparison analysis at their own expense to shorten the identification interval. The average time for identification of the remaining four cases through NamUs was 9.5 months. The charred and two decomposed cases were presumptively identified and confirmed based on familial DNA collected by law enforcement. Coordination with out-of-state law enforcement for DNA collection was required in two cases. Anthropologic analysis was essential for the identification of the remaining two cases in building an accurate profile within NamUs. In the first case, two fragments of bone were found in the woods, which, based on the anthropologic profile, were presumptively identified to belong to a woman who had gone missing in the area one year prior. She was positively identified based on familial DNA comparison previously submitted to NamUs at the time she had gone missing. The second case was a decomposed male found floating in a lake after significant flooding. The remains were identified as a missing person from another state nearly two years after the remains were found. A cold hit was received from NamUs after family members submitted DNA 14 months after the decedent was reported missing and 11 months after the remains were found.

These cases emphasize not only the multidisciplinary approach with transnational cooperation needed for positive identification but also the early entry of anthropologic and DNA data into the database. In 2017, Tennessee passed a law requiring that medical examiners and law enforcement enter all unidentified remains or missing persons cases into NamUs after 30 days. Currently, only five other states have similar laws. Although a great resource, the long wait times suggest the need for additional laboratory options to prevent further backlog as more states introduce legislation requiring the use of NamUs. In the cold hit case, the remains were not matched for nearly two years. This case highlights the importance of medical examiners, coroners, and families utilizing the database, as this family waited over a year after the decedent went missing to submit DNA. The success of the system is heavily dependent not only on medical examiners and coroners entering unidentified remains, but also the entry of missing persons cases by law enforcement as well as relatives of the decedents submitting reference samples. In conclusion, a transnational and collaborative approach with prompt and diligent case entry nationwide is needed to facilitate positive identification.

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### NamUs, Unidentified Remains, DNA Analysis

## H122 The Role of Postmortem Computed Tomography (PMCT) In High-Energy Traumatic (HET) Deaths

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**Learning Overview:** The goal of this presentation is to highlight the importance of a combined approach (postmortem imaging + autopsy) in the study of deaths due to HET that often result in heterogeneous, complex injury patterns with both organ and osseous injuries, including neurovascular structures. This study compared the PMCT and autoptic findings of 21 subjects who died due to HEG and according to the results (compared with those found in the literature), it was concluded that the two techniques (PMCT and autopsy) complement each other: PMCT is very useful for 2D or 3D documentation and analysis of fractures and pathologic gas collections (air embolism, subcutaneous emphysema after trauma, hyperbaric trauma, decomposition effects), while autopsy constitutes the method of choice for the detection of thoracic and abdominal visceral injuries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of understanding the many advantages provided by PMCT and 3D reconstruction (performed in addition to traditional autopsy) in the approach to deaths due to HET that currently represent one of the leading causes of death and disability (the sixth-leading cause of death and the fifth-ranking cause of moderate and severe disability); deaths resulting from “trauma” are some of the most common cases encountered by practicing forensic pathologists.

The increasing collective experience with PMCT and the collaboration between radiologists and medical examiners appears to be of great usefulness in the reconstruction of the course of events and in determining the cause of death. The idea of using imaging tools in forensic investigation emerged several decades ago and was followed by the concept of objective non-invasive documentation of body surface for forensic purposes. Postmortem imaging has evolved considerably over the years and is now a well-known and useful tool for forensic investigation.

In this study, the feasibility of the complementary use of PMCT with conventional autopsy in trauma victims was evaluated. Trauma, defined as any physical force or agent that causes bodily harm, is a worldwide pandemic and one of the leading causes of death and disability. Statistical data claim that about 5.8 million people die following, or from, traumatic events every year worldwide, which accounts for 10% of global mortality. In particular, HET, generally associated with road traffic accidents, collisions, falls from a great height, or crushing accidents, often results in heterogeneous, complex injury patterns with both organ and osseous injuries, including neurovascular structures.

A total of 21 subjects who had sustained various types of blunt, HET were selected from the Section of Legal Medicine at the University of Pisa in Italy. Before autopsy, a PMCT examination (Toshiba® Aquilion 16 CT scanner) was performed, and after the acquisition of the raw images, Multi-Planar Reconstruction (MPR) and Virtual Reality (VR) reconstructions were performed with dedicated software.

All the typical lesions were diagnosed by the two techniques, PMCT, and autopsy; nevertheless, there were some peculiar differences. For visceral traumatic lesions, most were detected by both techniques (autopsy and PMCT), such as those at cephalic extremity, thoracic organs, and abdominal wall. In contrast, certain lesions were recognized only by macroscopic section; these lesions included multiple brain contusions/brain lacerations, laceration of the pericardium, subpleural hemorrhages, diaphragmatic contusion, and complete bladder rupture. Lesions seen only at PMCT were pneumopericardium, pneumomediastinum, ground glass parenchymal opacities in the lungs, pneumothorax, diaphragm laceration, and pneumoperitoneum.

In conclusion, the two techniques (PMCT and autopsy) complement each other. PMCT is very useful for 2D or 3D documentation and analysis of fractures and pathologic gas collections (air embolism, subcutaneous emphysema after trauma, hyperbaric trauma, decomposition effects), while the classic autopsy documents better the various visceral lesions. PMCT is more sensitive than conventional autopsy in detecting skeletal injuries, while the autopsy constitutes the method of choice for the detection of thoracic and abdominal visceral injuries.

PMCT should be considered a useful tool in addition to conventional autopsy in evaluating trauma victims. It detects further bone fractures in body parts difficult to investigate during autopsy (i.e., posterior regions), facilitating the pathologist in the reconstruction of events and in determining the cause of death.

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### PMCT, Autopsy, Traumatic Death

## H123 The Role of Postmortem Computed Tomography (PMCT) in the Assessment of the Cause of Death in a Natural Disaster (Flooding): A Sicilian Experience

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**Learning Overview:** After attending this presentation, attendees will understand that PMCT is a useful tool to evaluate bodily injuries occurring during a natural disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that PMCT may be used as a single investigation to assess the cause of death in selected cases.

PMCT is a tool widely used in the forensic field and its applications focus mainly on cases of non-natural death such as floods, natural disasters, or whenever a crime is suspected. Sometimes it can be preferred to traditional autopsy, especially in cases where the bodies are heavily damaged. The main advantages are the ability to provide rapid and complete results on the main parenchymal changes and identifying foreign bodies, allowing a preliminary and sometimes definitive assessment of the cause of death. Here, the contribution of the PMCT images in the assessment of the cause of death of subjects who died during two natural disasters occurring due to flooding in Sicily, Italy, is reported.

The non-contrast whole-body thin slice PMCT scan of 48 bodies was performed: 37 subjects (16 female; 17 male; 4 children) died due to flooding that occurred in Messina; 11 subjects (4 female; 4 male; 3 children) died due to flooding in Palermo. Then the external examination of each body was provided.

In 28 cases, the CT investigation revealed the presence of slush in the airways and the cause of death was determined as acute asphyxia due to airway clogging. In 17 cases, the CT images showed severe crush injuries from debris scatter and the cause of death was assessed as polytrauma (head and/or thorax and/or abdominal trauma). In three cases, some body parts were found and in only one case was it possible to determine the cause of death as airway clogging. The external examination revealed severe injuries in a subject who died during the Messina flooding; no external injuries were evaluated in subjects who died in Palermo.

This report highlights the usefulness of PMCT in a natural disaster, in which the radiographic findings can lead to a definitive cause of death, even if a complete autopsy is not performed. Moreover, the images allow better evaluation of the different density of clogging fluid, the location of the fluid, and the lung parenchymal alterations. The PMCT, in selected cases, can be considered an important tool in determining the cause of death, leaving the bodies intact and shortening the time of the investigations.

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### Flood, Virtopsy, Mechanical Asphyxia





## H124 Conflict of Interest in the California Coroner or Sheriff-Coroner Systems

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the California coroner's, sheriff-coroner's, medical examiner's, and forensic pathologist's role in medical-legal death investigations and if any conflict of interest is perceived by these professionals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by determining if the beliefs of those currently working in the California death investigation systems indicate a potential for conflict of interest in coroner or sheriff-coroner systems compared to medical examiner systems.

**Introduction:** In California, there are 58 counties with a mixture of death investigations systems. Only four counties utilize a medical examiner system that is a separate office from law enforcement. Los Angeles County employs a medical examiner and a coroner system. There are four counties that utilize a coroner system and the remaining 49 utilize a sheriff-coroner system. In coroner and sheriff-coroner systems, a forensic pathologist or physician who is not trained in forensic pathology performs the autopsy and determines cause and manner of death, and the coroner or sheriff-coroner signs the death certificate. However, the coroner or sheriff-coroner can change the manner of death regardless of what the forensic pathologist determines in his or her death investigation. Because a separate medical examiner system is expensive and probably not warranted in counties with smaller populations and budgets, in these counties the duties of signing death certificates are given to the coroner or sheriff-coroner, elected officials, who do not need to be a licensed physician per California law.

**Methodology:** To collect the responses of professionals in the death investigation field, an electronic survey was created and emailed to coroners, sheriff-coroners, medical examiners, and forensic pathologists. Demographic information was obtained as well as the respondent's beliefs in regard to a potential conflict of interest, in general, in the case of officer-involved shootings and in-custody deaths or deaths at time of arrest. Statistical analysis was completed to compare the survey responses regarding conflict of interest and correlation with their job titles. Chi square analysis was also used with the demographic data collected from the survey to determine if there were any significant difference in these variables that may have impacted the opinions of the respondents.

**Results and Conclusions:** Of the 78 recruiting emails sent to email addresses collected, 35% were returned. California utilizes predominately sheriff-coroner systems, the majority of responses were from counties that employ sheriff-coroner systems. Medical examiner systems were not represented as much. Of the responses returned, there is no conflict of interest in coroner or sheriff-coroner systems. There was no correlation between age, gender, years in profession, and highest level of education and the respondent's opinions. Age and gender had significant different results indicating that these variables may have had an impact on the opinions regarding potential conflict of interest in the California death investigation systems.

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### Coroner or Sheriff-Coroner Systems, Death Investigation, Conflict of Interest

## H125 Pancreatic Subcapsular/Interstitial Hemorrhage Associated With Acute Systemic Hypoxia and Its Impact on Endocrine Organs

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**Learning Overview:** The goal of this presentation is to show the impact of acute systemic hypoxia on the endocrine organs by means of biochemical and pathological examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying additional morphological and pathological findings in regard to acute systemic hypoxia.

**Introduction:** Previous investigations have demonstrated that pancreatic subcapsular/interstitial hemorrhage was associated with acute systemic hypoxia, during which blood amylase secretion from the exocrine glands was elevated. However, the frequency at which acute pancreatic subcapsular/interstitial hemorrhage occurs under systemic hypoxia, histopathological changes in pancreatic tissues under a hypoxic state, and the impact of hypoxia on endocrine organs have remained unclear. Therefore, changes in the pancreas associated with acute systemic hypoxia were analyzed.

**Methods:** Among the autopsies performed in the laboratory (104 cases; median age at death, 63 years; range 6–96 years), those involving sharp instrument injuries (hemorrhagic shock:  $n = 11$ ), blunt injuries ( $n = 15$ ), fire fatalities ( $n = 23$ ), asphyxia ( $n = 32$ ), drowning ( $n = 11$ ), and acute cardiac deaths ( $n = 12$ ) were analyzed. The level of pancreatic interstitial hemorrhage for each case was then scored; blood insulin, glucagon, and glucose were measured. Apart from pathological changes, changes in the percentage of insulin- and glucagon-positive pancreatic islet cells were examined using immunohistochemistry. Given the frequent confirmation of pancreatic subcapsular/interstitial hemorrhage in previous asphyxia cases, a culture of rat pancreatic cells was used to conduct a hypoxic experiment (5% oxygen), analyze insulin messenger RNA (mRNA) expression, examine insulin secretion in the cultured cells, and observe microstructural changes under an electron microscope.

**Results:** Analysis of pancreatic subcapsular/interstitial hemorrhage according to each cause of death revealed that those who died from acute and subacute asphyxia experienced hemorrhage more often than those who died from other causes. Those who died from acute asphyxia had higher blood insulin levels (1.1–26.4  $\mu\text{IU/mL}$ , median 6.0  $\mu\text{IU/mL}$ ) than those who died from subacute asphyxia (0.3–6.4  $\mu\text{IU/mL}$ , median 0.9  $\mu\text{IU/mL}$ ) and other causes of deaths (0.3–24.4  $\mu\text{IU/mL}$ , median 2.0  $\mu\text{IU/mL}$ ). In contrast, no differences in blood glucagon concentrations and percentage of insulin- and glucagon-positive pancreatic islet cells were observed among the causes of death. The increase in blood insulin showed no relationship with glucose. The hypoxia experiment using cultured rat pancreatic cells showed that insulin mRNA expression became notable within 10min of hypoxia, while insulin concentration in the culture supernatant became elevated. Electron microscopy revealed mitochondrial swelling in the cultured rat pancreatic cells after 15min of hypoxia.

**Conclusion:** Pancreatic subcapsular/interstitial hemorrhage was observed more frequently in acute/subacute asphyxia cases, with insulin levels being higher in acute asphyxia cases than in cases of other causes of death. The results presented suggest that an acute/subacute systemic hypoxic condition affects mechanisms involved in pancreatic insulin secretion. These findings suggest that acute asphyxia increases serum insulin levels, at least in part, by promoting mechanisms involved in insulin secretion. The aforementioned results indicate that blood insulin secretion level could be an indicator of systemic hypoxia.

### Hypoxia, Ischemia, Endocrine

## H126 A Multidisciplinary Approach Is Mandatory in the Forensic Sciences

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**Learning Overview:** After attending this presentation, attendees will better comprehend the importance of a multidisciplinary approach in forensic science, particularly in solving not only complex cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing scientific data on the importance of the multidisciplinary approach in the management of forensic cases. Specifically, this presentation examines the importance of applying a multidisciplinary approach to not only complex cases; the combination of all evidence can lead to the correct reconstruction of the events.

The word forensic originates from the Latin word *forensis*, which means: public, to the forum, or public discussion. A modern definition of forensic is: relating to, used in, or suitable to a court of law.<sup>1</sup> Forensic sciences concern the application of scientific knowledge to legal problems, and they are vital tools in any legal proceeding.<sup>2</sup>

The forensic sciences, including forensic chemistry, forensic biology, forensic anthropology, forensic medicine, forensic material science, forensic engineering, computational forensics, etc., are broadly used to resolve civil disputes, to justly enforce criminal laws and government regulations, and to protect public health. Frequently, several crimes are classified as “cold cases” because they remain unsolved; in contrast, in other cases an individual may be convicted of a crime they did not commit, resulting in innocent subjects being condemned. These two undesirable situations could be the result of lost evidence during a criminal investigation.

In this presentation, a literature review was conducted; its aim was to highlight the importance of a multidisciplinary approach in solving cases in a forensic investigation. Particularly, this presentation highlights the importance of collaborating with different professionals in order to address all questions related to the so-called “cold” case. Moreover, the importance of a multidisciplinary approach should not only be to complex cases, as the combination of all evidence can lead to the reconstruction of the events.

Five case reports were selected: in the first case, a 31-year-old weightlifter, who presented to the emergency department due to an accidental fall that resulted in left thigh trauma was presented.<sup>3</sup> In Case 2, a suspected car accident was discussed.<sup>4</sup> Case 3 concerned the case of a 55-year-old man who was killed in front of his neighbor’s house.<sup>5</sup> Case 4 reports the case of a 55-year-old man found dead by his wife in his covered garage.<sup>6</sup> Finally, the last case discussed a 42-year-old male who died from unknown causes in his home.<sup>7</sup> In Case 1, the cause of death was due to necrotizing fasciitis as an unusual consequence of anabolic-androgenic steroid use. In Case 2, the identification of a fiber was fundamental to distinguish between an accident and a homicide. In Case 3, the disciplinary teamwork of several forensic experts clarified a complex dynamic of a homicide. In Cases 4 and 5, a multidisciplinary approach allowed the exact cause of death to be ascertained, excluding the hypothesis of sudden death. In Case 4, the cause of death was a fatal arrhythmia due to the cardiotoxic effects of cocaine consumption. In Case 5, it was determined that the cause of death was respiratory distress due to COVID-19 infection.

This cases series shows that in forensic cases, particularly in complex criminal cases, the use of a multidisciplinary approach is mandatory. This has to involve different specialists, depending on the cases, to guarantee a complete and correct scientific evaluation to support the investigation.

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### Forensic Sciences, Multidisciplinary Approach, Cold Case

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## H127 Trends in Postmortem Microbiome Research Activities: A Ten-Year Review

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**Learning Overview:** After attending this presentation, attendees will understand the advancements made in postmortem microbiome research over the past decade (2010–2020) with the goal of informing the development of scientific community standards important to future forensic practice. The postmortem microbiome is an emerging field in forensic science with broad application for death investigation (e.g., time since death, manner of death, and provenance). While the foundation for forensic microbiology began in the early 2000s, resulting from bioterrorism threats, the expansion of using entire microbial communities (the microbial organisms, their genes, and their gene functions) in a forensic context began in the early 2010s. Improvements in molecular (technical and computational) approaches over the past 20 years to study the microbiome in medical fields have revealed the power and potential use of microbial diversity as a means to explore the associations of microorganisms with human health of living individuals. Researchers within forensics adopted these technologies to determine if and how the postmortem microbiome could be used to aid death investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and practitioners by reviewing the sequencing technologies used by forensic researchers since 2010 until the present day. These technologies and analytical approaches will include, but are not limited to, 16S ribosomal RNA (rRNA) gene-based amplicon sequence analysis and whole genome shotgun metagenomic analysis.

A summary on the studies in postmortem human microbiome research activities will be presented. Initially reviewing the early history of forensic microbiology, this analysis will focus on the body of work that describes the postmortem microbial community compositions under a variety of death circumstances. Specifically, this presentation will describe the work completed as laboratory studies, longitudinal assessments, and surveys of routine autopsy cases, which highlights the variation and complexity of postmortem microbiomes, as well as transcriptomes of the microbiomes. Further, information regarding technical needs, funding support, and knowledge gaps that remain for this field will be covered.

The interest in high-throughput sequencing and other “-omics” technology, coupled with the ever-improving accessibility, affordability, and user-friendly analytical capacities, for forensics does not appear to be waning. Rather, an uptick in the use of postmortem microbiomes for assisting during death investigation seems inevitable. Yet, as a scientific community, standards must be established; analytical approaches, models, and data interpretation validated; error rates confirmed; and the robustness of these technologies for the field of forensics evaluated. The results from this review are important to be able to advance the field of postmortem microbiomes for forensic applications and for the outcomes of this research to be able to eventually progress to microbiome-based evidence for routine use in casework.

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### Forensic Science, Postmortem Microbiome, Death Investigation

## H128 Conceptualizing the Gut Thanatomicrobiota in Substance Abuse Disorders

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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 transverse colon samples from Italian cadavers. Specifically, attendees will learn methods to assess the microbial diversity after death using 21 overdose and 19 natural death cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting new information on the community composition of postmortem microbial colonizers in drug overdose cases, which could be useful to investigators to devise better methods and improve the accuracy of estimating the cause and time of death using an underutilized molecular method. By probing the relative abundances of specific microbes present in the transverse colon, a comparative analysis was used to identify a possible link between commonly abused drugs and microbes present during the decomposition process.

In this century, drug abuse continues to be a national crisis. Since 1999, the number of opioid-induced overdoses has increased four-fold to more than 500,000 deaths. The microbiota gut-brain-axis is a bidirectional circuit that links the neural, endocrine, and immunological systems with gut microbial communities. Gut microbiota play significant roles in human mind and behavior, specifically pain perception, learning capacity and memory, mood, and emotion, and anxiolytic effects and temperament. Also, disruptions in the gut microbiome have been associated with substance use disorders. While much research still needs to be performed, elucidating the interplay of gut microbiota in substance abuse disorders may produce promising avenues for future forensic development. The goal of the current study was to determine gut microbiome composition in substance abuse disorder cases using transverse colon tissues of 21 overdose criminal cases versus 19 non-overdose-related cases. The hypothesis was that postmortem samples of the same origin will reveal similar taxonomic relationships. Using weighted UniFrac analysis, drug abuse was found to be a significant factor in determining microbiome similarity ( $F = 1.93$ ;  $df = 1, 35$ ;  $p < 0.048$ ;  $R^2 = 0.05$ ) indicating that there are detectable differences in composition that are attributable to substance abuse. Using unweighted UniFrac, however, sex was instead found to be a significant predictor of microbiome similarity ( $F = 1.88$ ;  $df = 1, 30$ ;  $p = 0.028$ ;  $R^2 = 0.05$ ). A heatmap was generated of the relative abundances of the 30 most prevalent bacteria per case and their associated substance profile. The results revealed that samples of the same origin cluster together, showing a high degree of similarity between samples and a low degree of similarity among samples of different origin. This examination of human transverse colon microflora in decomposing cadavers expands the emerging literature on postmortem microbial communities, which will ultimately contribute to advanced knowledge of putrefaction.

### Thanatomicrobiota, Cadaver, Gut



## **H129 Decontamination Strategies for Sampling Internal Bone Microbiomes Submerged in Aquatic Environments**

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**WITHDRAWN**

## H130 Caspase 9 and Caspase 3 Immunohistochemical Reactivity Pattern in Skeletal and Cardiac Muscle at Different Times After Death: A New Tool for Postmortem Interval (PMI) Determination?

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**Learning Overview:** After attending this presentation, attendees will understand the importance of the identification of new markers that could be used as possible PMI indicators, especially in contexts in which the reliance on the classic thanatochronological triad—livor, rigor, and algor mortis—is not conclusive.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the relationship between the activation pattern of caspases 9 and 3 on skeletal and cardiac muscle samples and PMI.

The determination of the amount of time elapsed from one's death to the recovery of the body—the so-called PMI—has always relied on the evaluation of three main consecutive thanatological phenomena: livor, rigor and algor mortis. Despite its remarkable value, several conditions exist, such as premature decomposition, postmortem scavenging, extreme environmental conditions, in which this classic triad shows some limits, thus proving not conclusive in terms of PMI estimation. Such a reflection thus led to the evaluation, with a semi-quantitative approach, of the immunohistochemical reactivity pattern of two apoptosis mediators, caspase 9 and caspase 3, on samples of skeletal and cardiac muscle obtained from sacrificed rats at different times after death.

For this purpose, 23 male C57BL/6J rats were sacrificed by means of chloroform inhalation, and samples of quadriceps femoris and left ventricular wall were collected at 0, 4, 8, 12, 24, and 72 hours after death. All samples were fixed in formalin, then embedded in paraffin blocks for light microscopy. Ten sections were prepared from each sample, nine of which assessed for the immunohistochemical reactivity to caspases 9 and 3, the remaining one stained with hematoxylin-eosin in order to study the tissue morphology.

Both in skeletal and cardiac muscle, a slight increase in caspase 9, but not caspase 3, immunoreactivity was detected 4 hours after death. Caspase 9 immunoreactivity kept increasing up to 12 hours after death; a slight caspase 3 immunoreactivity appeared 8 hours after death, and further increased after 12 hours, although lower than caspase 9 reactivity. Such a pattern is consistent with a primary activation of caspase 9, which in turn activates caspase 3, the “executioner caspase” responsible for the apoptotic degradation of the cell molecules. A general decrease of immunoreactivity—though the caspases 9 and 3 relative pattern was maintained—was observed at 24 hours, followed by the absence of caspase 3 and just a moderate caspase 9 immunoreactivity 72 hours after death. Such general decrease ran parallel with the morphologic changes the muscular tissue underwent, consisting of a gradual, increasing alteration of its architecture at 24 and 72 hours.

The present results indicate that both skeletal and cardiac muscular tissue maintain metabolic activity up until 72 hours after death. Within this lapse of time, there is a gradual variation of the pattern of caspases 9 and 3 immunoreactivity—a first increase up to 12 hours after death, when the muscular tissue's architecture is still maintained, followed by a decrease up to 72 hours, as the degradation processes go on—observed, thus making it possible to consider both apoptotic mediators useful immunohistochemical markers for PMI estimation.

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### PMI, Caspase 9, Caspase 3

## H131 Maximum DNA Recovery From Cold Case Victims Using Ancient and Forensic Extraction Methods

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**Learning Overview:** The goal of this presentation is to compare quantitative Polymerase Chain Reaction (PCR), Short Tandem Repeat (STR), and preliminary whole-mitochondrial genome results from the skeletal remains of cold case victims from Maricopa County, AZ, using comparative DNA extraction techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees about the results of comparisons of different methods for DNA recovery for Combined DNA Index System (CODIS) marker and mitochondrial genome analyses in the context of degraded DNA.

Human identification using DNA analysis is a central focus of forensic science. However, environmental, microbiological, and climatic factors often limit the amount of recoverable DNA (i.e., short DNA fragments and nucleotide damage) following death, especially in circumstances where only hard tissues, such as bones and teeth, are available for study. Despite these limitations, the field of ancient DNA (aDNA) analysis has advanced DNA methodology remarkably, allowing for the rapid quantification and genome-wide reconstruction of ancient samples (e.g., human, megafauna, and pathogen) dating as far back as the Middle Pleistocene (781,000 to 126,000 years ago). As a result, the applications of aDNA techniques to forensic cold case victims using skeletal material recovered from harsh, desert environments from Maricopa County, AZ, have the potential to recover the complete genetic profiles of these previously unidentified victims. These individuals remained unidentified because traditional attempts failed to produce adequate DNA yields. This study examines the results of quantitative PCR, STR, and whole-mitochondrial genome analyses generated from two different DNA extraction techniques used to recover DNA from the skeletal remains of cold case victims from Maricopa County, AZ.

In collaboration with Arizona's Maricopa County Office of the Medical Examiner, this study obtained 75 bone and tooth samples representing 75 open and/or missing persons cases. This study obtained DNA from these victims using two different DNA extraction methods: one devised by Dabney and colleagues (ancient DNA) and another by Loreille and colleagues (forensic DNA). Absolute DNA concentrations were measured using the Quantifiler<sup>®</sup> Trio DNA Quantification Kit, and STR profiles were generated using Promega's<sup>®</sup> PowerPlex<sup>®</sup> Fusion 6C System, a kit designed to amplify the expanded CODIS core loci (i.e., 23 autosomal STRs, 3 Y-chromosomal Short Tandem Repeats (Y-STRs), and amelogenin). In addition, this study built double-stranded DNA libraries and performed a long hybridization (48 hours) targeted enrichment using mitochondrial RNA baits. Libraries were pooled and sequenced on an Illumina<sup>®</sup> MiSeq<sup>®</sup> platform, and haplogroups were called using an in-house custom mitochondrial DNA computational pipeline. The analysis suggests that aDNA extraction methods yield higher quantities of DNA for cold case identification than traditional forensic DNA extraction methods. The integration and adoption of optimized aDNA protocols, such as the Dabney DNA extraction protocol, in forensic case analyses may improve the success rate of acquiring adequate STR profiles and whole-mitochondrial genome assembly to identify cold cases in circumstances where traditional extraction techniques have failed.

**Genetics, CODIS, MtDNA**





## H132 Molecular Testing in Sudden Death Associated With Epilepsy in a Forensic Office: Genotype-Phenotype

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**Learning Overview:** The goal of this presentation is for attendees to understand the role of molecular testing in epilepsy death investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the understanding of the value of molecular testing in determining cause and manner of death.

Epilepsy increases the risk of sudden death and presents significant challenges for the certifying medical examiners. Molecular genetic methods in conjunction with standard Neuropathologic (NP) evaluation in a cohort of 65 non-traumatic, non-alcohol-related seizure cases were undertaken. These were selected over a four-year period from among those with “Epilepsy” or “Seizure” on the death certificate, and/or submitted for NP evaluation; subjects were aged 2 weeks to 47 years (median, 22.6 years), and there were 37 males, 27 females, and 1 transgender female. Molecular analysis of a panel of 132 genes, associated with dominant epilepsy syndromes or neurodevelopmental disorders with epilepsy, was performed. NP features under analysis comprised End-Folium Sclerosis ([EFS]; neuronal loss and gliosis, CA4), Mesial Temporal Sclerosis ([MTS]; neuronal loss and gliosis, CA4 and CA1/CA3), hippocampal dysgenesis (macroscopic asymmetry and Dentate Gyrus [DG] abnormalities), and cerebral dysgenesis (e.g., abnormal cortical gyration, callosal agenesis, heterotopia, hamartia).

Pathogenic or likely pathogenic variants were found in three (4.6%): (1) a frameshift variant in *CACNA1H*, with MTS; (2) a nonsense variant in *SCN1A* (Dravet Syndrome), with EFS and subarachnoid heterotopia; and (3) a splice-site missense variant in *SCN2A*, with EFS. Twenty-two (33.8%) had Variants of Uncertain Significance (VUS): two had cerebral dysgenesis (*GRIN2B*; *GLI2*, *GLRA1*, *KMT2D* in a decedent with trisomy 21); and ten had EFS, with or without other NP (*CDKL5*, *CHD2*, *SCN9A*, *GFAP*, *LGII*, *GLI2*, *FLNA*, *HCN1*, *SPTAN1*, *NSD1*, *FLNA*, *HCN1*, *CRYAB* P AR variant carrier, *DSC2*). One carrier of a TBCD loss-of-function variant had EFS, focal cortical dysplasia IIIA, and cerebellar polymicrogyria. Four with VUSs had no NP findings. The remaining 40 subjects (61.5%) tested negative, among them three with hippocampal dysgenesis, one with Doose syndrome and DG bilamination, and one with Lennox-Gastaut syndrome and cerebral dysgenesis.

In summary, this preliminary cohort of non-traumatic epilepsy deaths with molecular testing identifies mutations that contribute etiologically specific information impacting both death certification as well as potential care of surviving family members.

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### Epilepsy, Sudden Death, Molecular Testing

## H133 The Sound of Violence: The Utility of Voice Recording in the Investigation of the Cause of a Sudden Death in Custody

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**Learning Overview:** The goal of this presentation is to present the results of the investigation of a sudden death in custody occurring in a forcefully restrained suspect, which was ruled as an accident due to methamphetamine toxicity and coronary artery disease after an autopsy. Audio evidence and body cam footage was used to establish a sudden change in breathing sounds and rate after the decedent's mouth and nose were obstructed by jail personnel who were attempting to remove jewelry from the decedent's ears.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in two ways: (1) by demonstrating a counterfactual approach to assessing cause of death during forceful restraint by comparing the fatality risk of methamphetamine and asymptomatic coronary artery disease to the risk from three minutes of airway obstruction by a gloved hand, and (2) by illustrating the use of audio recording analysis to demonstrate airway obstruction that preceded cardiorespiratory arrest.

The case involves a 32-year-old male known methamphetamine user who was arrested for public intoxication and brought into custody. Several intake officers attempted to remove his earrings while he was restrained in a supine position. The man spat at the officers and one officer's gloved hand immediately covered his mouth. Body camera footage was then obstructed until three minutes later, when the man had stopped breathing and turned blue. After a few minutes, unsuccessful resuscitation efforts commenced.

The officers' reports all denied obstructing the man's airway, claiming that the man's shirt was held one inch away from his nose and mouth as a makeshift "spit-guard."

Autopsy revealed intramuscular hemorrhage secondary to Blunt Force Trauma (BFT) to the back, moderate to severe obstruction of the left anterior descending coronary artery, and a blood methamphetamine level of 1.85mg/L. Despite evidence of BFT and a history potentially consistent with asphyxia, the cause of death was concluded to be due to the toxic effects of methamphetamine combined with coronary artery disease.

Although body cam video was obstructed, the breathing sounds and moans the man was making were captured on audio recording. This allowed for quantification of the man's breathing rate, which paradoxically dropped from 55bpm prior to the spitting incident to 20bpm upon his last breath, a finding that is inconsistent with a methamphetamine-related death, characterized by hyperventilation until cardiac arrest.

To the naked ear, the moans became muffled immediately after the spitting incident, and thus a phonetic computer program was used to quantify the sound frequencies associated with the man's vocalizations before and after the spitting incident.<sup>1</sup> The post-spitting spectrogram was missing the higher frequencies that were present in the pre-spitting spectrogram. An ad hoc experiment was conducted of a 30-year-old man moaning with and without a hand over his mouth, and the spectrogram of the obstructed scenario was identical to the post-spitting spectrogram of the decedent.

A final step of the analysis was to estimate the competing risks of sudden death from the causes listed in the autopsy report: methamphetamine toxicity and coronary artery disease. The estimated risk of death due to methamphetamine toxicity at a recreational dose is less than 1 per 353,000 doses of methamphetamine.<sup>1</sup> The risk of sudden cardiac death in adult men aged 31–35 years like the decedent is 1 in 31,250 annually.<sup>2</sup> Assuming the decedent was in the least healthy 10% of the population for cardiac health, his annual risk of sudden cardiac death would be 1 in 3,125, and the risk during the three-minute interval preceding his death would be 1 in 548 million. While it is possible that the left anterior descending coronary artery obstruction and/or methamphetamine interacted with the asphyxia to result in a fatal tachyarrhythmia, the degree to which this occurred is unquantifiable.

The result of the analysis was used to conclude that the largest single factor contributing to the man's death was asphyxia by manual smothering, with coronary artery disease as a likely secondary cause, and methamphetamine presence only a possible contributing cause. The manner of death was a homicide.

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2. Bagnall, et al. A prospective study of sudden cardiac death among children and young adults. *N Eng J Med*. 2016;374:2441-52.

### Death in Custody, Audio Recording Analysis, Counterfactual Causation

## H134 Survival Intervals Following Fatal Single Gunshot Wounds of the Head

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**Learning Overview:** The goal of this presentation is to elucidate factors associated with a survival interval following an ultimately fatal gunshot wound of the head.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that not all fatal gunshot wounds of the head are immediately fatal and by delineating the factors associated with survival.

Forensic pathologists are commonly asked to opine, based on autopsy findings, about the amount of physical activity or length of survival following gunshot wounds. Standard forensic pathology teaching is that gunshot wounds of the central nervous system are immediately incapacitating/fatal while other injuries, even a perforating gunshot wound of the heart, may not be. These opinions may help corroborate or contradict witness statements or help explain scene findings.

The Connecticut Office of the Chief Medical Examiner is a single-facility, centralized, state-wide medical examiner system serving a population of approximately 3.6 million people with 29,000–31,000 deaths per year during the span of this study. It receives all reports of jurisdictional deaths, including all gunshot wound fatalities. There were 3,081 fatal gunshot wound deaths over this 20-year review, of which gunshot wounds of the head were involved in 1,769. Of these, there were 62 deaths due to a single gunshot wound of the head in which the person survived from 6 to 336 hours (14 days). The mean survival interval in these cases was 57 hours. The manners included 35 suicides, 24 homicides, 1 accident, and 2 undetermined. Fifty-six decedents were male (90%) and the ages ranged from 13–90 years (mean: 36 years). The firearms included: 33 handguns, 3 rifles, and 26 unknown. The bullet exited in 26 instances. The wound involved both cerebral lobes in 43, and involved the brain stem in two. Penetration/perforation of only one cerebral lobe occurred in 15, and 3 wounds were midline. Skull fractures were described in 37 (60%). Range of fire was: 33 contact, 12 close, and 16 undetermined. Projectiles included: 10 small caliber (3 rifles with .22), 15 medium caliber (.32, .38, 9mm), and 4 large caliber (.40 and .45). Eight decedents had expanding hematomas (subdural and/or epidural) described. Three patients, all with self-inflicted wounds, were reported to be fully conscious when discovered. The first had an entrance wound under the chin and an exit wound inferior to the eye (the projectile did not enter the skull). Death was caused by aspiration of blood from the gunshot wound complicated by metastatic lung cancer. The second involved the superficial parietal lobes. The third had a facial entrance wound with perforations of the left frontal and right parietal lobes and an expanding subdural hematoma. All these patients were speaking when discovered; only the first remained fully conscious on arrival to the hospital (two projectiles were deformed, jacketed bullets, and one was a jacketed .25 bullet).

Opinions of forensic pathologists regarding these survival intervals must distinguish between incapacitation and death. A single gunshot wound of the head normally causes immediate incapacitation, but rarely there may be a period of consciousness and even a prolonged survival interval, particularly with prompt medical intervention. This may occur with gunshot wounds that involve both cerebral lobes and in the context of a variety of calibers and ranges of fire. The mechanism of death due to gunshot wounds of the head typically involves disruption of the central nervous system and may include hemorrhage and expanding intracranial hematomas. The neurological trauma almost always results in immediate cessation of brain function, but cardiopulmonary function may persist or return with resuscitation attempts. Rarely, a period of brief consciousness may occur even when a projectile penetrates the brain. When offering these opinions, it is best to interpret the autopsy findings in context of the circumstances.

### Forensic Pathology, Gunshot Wounds, Survival Intervals

## H135 Experimental Shots in Tissue-Simulant Materials and Analysis Through Computed Tomography (CT) Scanning

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**Learning Overview:** After attending this presentation, attendees will understand the contribution of modern wound ballistic experimentation in providing crucial information regarding bullet paths within the human body.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of experimental shootings in tissue-simulant materials, which is useful when handling complex cases.

Projectile paths through the human body and subsequent injuries may not always be predictable as they depend on a variety of factors, such as impact velocity, geometrical shape of the bullet, shooting distance, incidence angle, and affected tissue.

Ballistic gelatin materials were used as tissue simulants. Experimental shots were performed with various pistol and revolver types (9mm Parabellum®, .45 Automatic Colt® Pistol [ACP], .357 Magnum®, etc.) and with various bullet types (full metal jacket, hollow point). Furthermore, shots with rifles were conducted in 7.62 Kalashnikov and 5.56mm NATO calibers. Fired blocks were scanned by a Computed Tomography (CT) scanner thus allowing calculation of the density of entry/exit wound morphology as well as the bullet path deviation, according to the shooting line.

The analysis provided an opportunity to observe and calculate the characteristics of wound channels in a highly accurate numerical analysis and to evaluate the effectiveness of the used projectiles. The created wound paths represent the amount of energy that is transferred into the human body, which in turn can often be associated with the traumatic results. Density of the gelatin blocks was measured on average at 1.03g/ml.

Kinetic energy was measured and permanent cavities were determined by CT. A proportionality between the kinetic energy deposited in the used blocks and the resultant cavities could be visible. These experiments may prove quite useful in aiding routine forensic pathology practice as they document a bullet's path through the human body, thus providing invaluable confirmation of the autopsy findings.

Any subsequent effort to reconstruct the shooting using ballistic gelatin is useful in wound ballistic studies and enables us to document and record the wound track that a projectile follows in a simulation of the human body. The application of CT technology renders the objective easier, as any deflection, the total path length, and the final resting point of the projectile can be observed with great accuracy. Among the benefits is the calculation of the density of the gelatin used and the fact that the data can be recalled in the future for comparisons between them or for further analysis.

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### Forensic Science, Ballistic Gelatin, Experimental Shootings

## H136 Veterinary Forensic Pathology: Cases From an Emerging Discipline

*Beverly J. McEwen, DVM, PhD\*, Guelph, ON NIC 1B8, CANADA*

**Learning Overview:** The goal of this presentation is to inform the medical forensic community of the link between animal cruelty and interpersonal violence or people with psychological issues with examples of animal abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that patterns and types of trauma in animals are similar to human victims and by informing attendees of the link between animal cruelty and interpersonal violence.

Animal abuse and neglect may involve a single animal and one person or hundreds of animals and many people. Animals and people are victims of the same types of fatal injury and severe neglect; however, the anatomy and physiology of different animal species and even breeds of animals represent a unique challenge for veterinary pathologists. Yet, animal abuse rarely occurs in isolation—when animals are abused, people are at risk and when people are abused, animals are at risk.<sup>1</sup> Animals may be sentinels for domestic abuse and cruelty to a pet is a recognized mechanism of psychological control over a partner: no species of animal is immune to these crimes. The link between animal abuse and concurrent or predicted interpersonal violence is well established. Medical examiners, investigators, and attorneys may not be aware that veterinary pathologists are employed at universities and diagnostic laboratories to perform postmortems or that some have developed expertise in the pathology of animal abuse and neglect. These people should also be aware that animal abuse affects not only the animal, but individuals, families, and society. Veterinary pathologists in this emerging subspecialty are in the fortunate position of learning from examples of systemic failings in medical forensic pathology, such as inadequate oversight, training, and certification. They can also benefit from the recent advances in several jurisdictions to rectify these issues.

Internationally, submissions of animals to veterinary diagnostic laboratories from law enforcement agencies have dramatically increased over the past 15 years. Reasons for this are speculative but factors likely include changes in legislation and mandatory reporting of suspected animal abuse by veterinarians. Cases submitted to a university veterinary diagnostic laboratory by law enforcement agencies for postmortem examination will be used to illustrate examples of animal cruelty and the context in which they arose.

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**Forensic, Pathology, Veterinary**

## H137 Canid Predators Kill Bites and the Injuries to Their Animal or Human Victims

*Nanny Wenzlow, DVM, PhD\*, Université de Montreal, Canada ; William F. Hamilton, MD, District 8 Medical Examiner, Gainesville, FL 32608*

**Learning Overview:** The goal of this presentation is to illustrate the challenge and confusion that pet owners, law enforcement, and veterinarians or medical examiners may face when presented with a canid predation case as they can resemble animal cruelty and non-accidental killings (animal cruelty) or homicides. Proposed is a systematic postmortem examination of the injuries caused by canid predator kill bites.

The postmortem exams result from a series of deceased cats, dogs, and one human killed by canids (coyote or domestic dog) and will be presented with emphasis on specific characteristic injury patterns resulting from those bites. A thorough injury exam of the victims will allow the distinction between canid kill bites and non-accidental (cruelty)/homicidal injuries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by a case series illustrating the importance of trained veterinary forensic pathologists who carefully examine deceased animal bodies or body parts in order to determine accurately the cause and manner of death that allows distinguishing whether death was caused by a human through acts of cruelty or by a wild animal predator as part of his hunting behavior for feeding. For the human victim, it is important to notice that in this case the circumstances provided evidence of dogs in the presence of the victim prior to and at the time of death; however, if that information had not been available, the bite injuries on the deceased human body resembled stab wounds and could have led an unexperienced pathologist and investigators to the conclusion of homicide by stabbing.

No criminal investigation was pursued in any of the cases described as human interference and cruelty was excluded. The focus was now directed toward the management of free wild canid predators in urban settings for the here-described animal victims and demonstrated once again the importance of adult supervision of children in the presence of domestic dogs.

Multiple deceased dogs and cats were found in different geographic urban locations in Florida, Texas, and Ontario, Canada. Suspicious presentation of the bodies led law enforcement to initiate a death investigation, as foul play in the form of animal cruelty and non-accidental killing (homicide equivalent) was suspected. In some cases, veterinary clinicians in private clinics had been consulted by law enforcement prior to submitting the bodies to veterinary pathology and these clinicians had erroneously voiced the opinion that the lesions and mutilations had been caused by humans, aggravating the anguish of owners and investigators for the possibility of an active psychopath in their area. It is known and well established that animal cruelty acts are a deviant behavior in humans that is likely to escalate into child and domestic abuse and evolve to possible future serial killer behavior.

The cat victims were found either as half bodies or with large abdominal openings and most internal organs absent. The bodies, as well as the surrounding recovery site in urban locations, lacked obvious bloodstains and led investigators to conclude that: (1) the site of the deadly incident had been elsewhere, (2) the perpetrator was likely a human, and (3) the use of large tools had caused the sustained injuries. The dog victims were found without obvious external wounds and only some-to-no bloodstains in either suspicious locations or after a canine police unit had raided a property with multiple other dogs.

All animal victims were submitted for forensic necropsy to a trained veterinary forensic pathologist. The skin wounds of the cat bodies were often free of bloodstains and were lacerated with irregular, mildly hemorrhagic edges. The adjacent fur was arranged in wet streaks that had partially dried and was consistent with saliva from the attacking predator. Foreign fur was occasionally adherent to the front or hind claws of some cats. Dog and cat bodies showed in most cases the typical deep soft tissue crushing wounds as well as pharyngeal-tracheal hemorrhages in the ventral neck area with or without associated skin perforations. These lesions are typical for kill bites by canid predators. Some cat victims exhibited additional hyperflexibility of the proximal cervical vertebrae surrounded by associated hemorrhages, with subdural spinal hemorrhages—lesions consistent with prey shaking.

Asphyxiation due to these kill bites was the mechanism of death in most cases. Coyote DNA was isolated from the skin wounds of two cases. Human interference and cruelty was excluded in all cases and the manners of death were natural.

A 6-year-old boy was left unsupervised with two pitbull dogs in his aunt's yard. The victim was also known to the Department of Children and Family (DCF) for his highly aggressive behavior toward humans and animals, including killing neighborhood cats for the past two years. Later, the boy was found lifeless by his aunt; he had multiple skin lacerations and a nearly completely decapitated head. His death was reported to the medical examiner in accordance with Florida Statute 406.

A complete forensic autopsy was performed by the assigned chief medical examiner. The decedent's head remained attached to the trunk by a few slender skin bridges. Much of the scalp was torn away from the calvarium. The neck organ block had penetrating injuries to the larynx, and the mid cervical trachea was transected as was the adjacent esophagus and mid cervical spine. Randomly all over the body were numerous deep linear puncture wounds resembling stab wounds with deep penetrating injuries over the right shoulder, the back and lower trunk, and numerous superficial linear skin wounds on the head, neck, trunk, and the extremities, as well as minor superficial skin erosions occasionally associated with small superficial bruises on the extremities.

The probable cause of death was extensive canine kill bites with shaking and the manner of death was an accident.

As the child victim exhibited previous cruelty behaviors towards cats, one could speculate that his aberrant behavior was escalating from cat to potential dog killing and that his death could have been the consequence of a "self-defense" or counterattack by the dogs.

### **Dog Bites, Veterinary Forensic Pathology, Bite Injuries**

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## H138 Potential Applications of Micro-Computed Tomography (Micro-CT) in Forensic Casework: Evidence From Zebrafish

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**Learning Overview:** After attending this presentation, attendees will understand the differences in analytical power of traditional histology as compared with a 3D form of histology, X-ray histotomography, and be able to describe potential applications to forensics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence from zebrafish of the utility of X-ray histotomography and the potential value of this new imaging modality in future forensic casework.

Through zebrafish modeling, 3D tissue reconstruction data collected will support translation of utility of histotomography for use on human tissue in forensic casework. Histotomography is a form of micro-CT customized for tissue. Histology involves inspection of a small number of slabs of tissue that represent on the order of 1%–2% of the tissue volume, while histotomography potentially captures 100%. Potential applications of histotomography to forensic pathology casework will be considered, based on presented data from zebrafish pilot studies.

Traditional histology has been the standard tool for microscopic analysis of tissues in forensic pathology. The observed tissue architecture allows us to infer important aspects of cellular processes relevant to causes of death.<sup>1</sup> However, histology is destructive of the sample, limited in its value considering limited sampling and inability to provide volumetric data. 3D reconstructions from serial sections are impractical due to tissue distortion associated with cutting and floating sections on water.<sup>2,3</sup> Spatial patterns potentially relevant for inflammatory response or evaluation of vasculature are not fully discernable from a 2D image. In contrast, micro-CT does not require sectioning. Instead, minimally invasive punch biopsies can be rapidly collected, with or without guidance from lower resolution whole-body scans.

In this study, investigation of phenotypic detail with synchrotron based micro-CT was conducted using zebrafish as a model organism. Zebrafish were chosen because they are a small vertebrate with diverse tissues. Synchrotron, and potentially, institutional micro-CT, with the latest technologies, permits resolutions roughly equivalent to that obtained in digital slide scanning using a 20x lens, or ~1 micron optical resolution.<sup>3</sup> Whole zebrafish were stained in Phosphotungstic Acid (PTA) for contrast enhancement and embedded in polyamide tubing.<sup>4</sup> Samples were then scanned by synchrotron-based micro-CT. Projections collected from scanning were reconstructed that permitted observation of the whole organism through digital slices in three different planes. Reconstructions also allowed for 3D volume renderings or segmentation of different cell types, permitting discrimination of the larger, often branching structure of complex 3D tissue structures, such as vessels, that cannot be ascertained from just a few 2D histology images. Dynamic reslicing, particularly with technology like virtual reality, permits the analyst to study different cross sections of the sample without tissue destruction.

3D reconstructions obtained from experimental samples show detail across millimeters of tissue volume that is unattainable with traditional histology. Whole organism reconstructions can be viewed slice by slice on the web or with virtual reality. Such preliminary studies offer promise for translating this methodology to alternate sample specimens such as human tissue. Histotomography may be applied to forensic cases involving gunshot residue powder patterns, decomposition, trauma with sharp or blunt objects, or sudden death cases suspicious for structure-based or cellular-based disorders where total tissue architecture would be of value. Currently, a joint preliminary study is in development with the Dauphin County Coroner in Pennsylvania to scan human tissues sampled via punch biopsy from valid forensic casework.

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### Micro-CT, Forensic Pathology, Zebrafish

## H139 Personal Identification Using Part-to-Part Comparison of the Third Lumbar (L3) Vertebra From Antemortem and Postmortem Computed Tomographic (AMCT and PMCT) Scans

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**Learning Overview:** After attending this presentation, attendees will have gained a deeper understanding of the usefulness of part-to-part comparison of anatomical features comparing AMCT and PMCT.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of the initial findings of an ongoing study utilizing part-to-part comparison of the lumbar vertebra for personal identification. This presentation will examine the real-world application of using CT-derived 3D models of the L3 vertebra of AMCT scans of known individuals and compare them against true PMCT scans to establish the validity of using this technology for personal identification.

The confirmation of identification for an unknown individual is a critical part of forensic practice. The comparison of antemortem and postmortem imaging for the purposes of personal identification is a common tool in a variety of techniques such as odontology and other radiographic comparisons. An earlier pilot study was conducted as a proof of concept using antemortem and simulated postmortem 3D-rendered lumbar vertebra comparisons as a means for identification of an unknown individual.<sup>1</sup> The current study utilizes AMCT scans of known individuals and compares them against true PMCT scans of the same individuals.

The University of Leicester, East Midlands Forensic Pathology Unit utilizes PMCT extensively as part of their daily forensic practice. As part of this ongoing project, Leicester acquired ten matching antemortem scans for individuals that passed through their facility for PMCT scanning. The University of Leicester anonymized the scans so researchers at the University of South Florida were blinded to the identities of the AM and PM scans. Each scan was imported into the Mimics Innovation Suite v. 24 (Materialise) for 3D modeling. The L3 vertebra was then isolated and modeled via segmentation and thresholding. Each series of ten AM vertebra were registered with a target PMCT-derived vertebra. A part-to-part comparison was conducted for each vertebra, and a percent match was measured. A threshold of +/- 1mm was set for the part comparison. Every unknown PMCT L3 was correctly matched to the corresponding AMCT L3, signifying complete accuracy for this sample.

A Receiver Operator Curve (ROC) curve was calculated to determine 100% sensitivity and specificity with a cutoff point of 73.5% percent match. True identifications had an average percent match of 94.8% +/- 5.6%. Negative identifications had an average percent match of 35.7% +/- 11.4%.

The results of this study indicate a positive correlation with using part-to-part comparison for identification purposes. The next phases of this study will examine all lumbar vertebrae from L1 through L5. Additionally, future directions will examine other regions of the body as well as other imaging modalities. With the increased usage of PMCT, there is an equal increase in the availability and opportunity to utilize 3D tools, such as part-to-part comparison, for the successful identification of unknown individuals in a forensic setting.

*This study is currently funded by the National Institute of Justice Grant 2019-DU-BX-0031.*

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### Postmortem CT, Disaster Victim Identification, Lumbar Vertebrae



## H140 Imaging Impact: Can Computed Tomography Fractography Determine Direction of Fracture Propagation?

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**Learning Overview:** Fractography is the study of the surface features of a fracture. After attending this presentation, attendees will understand how fractography techniques derived from materials science can be applied to the study of bone. Evidence will be presented to demonstrate that Computed Tomography (CT) fractography can determine the point of impact of a bending force sufficient to fracture a bone and the direction of fracture propagation. The primary aim of this study was to assess whether CT fractography can determine impact site with a similar degree of accuracy to the direct examination of bone. A worked example of how CT fractography can be performed and utilized in a case of fatal pedestrian vs. vehicle trauma will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing attendees the theory and evidence behind CT fractography and by using illustrative examples, see how it can be used in routine forensic practice to improve the accuracy of fracture analysis.

**Background:** Bending forces are the most commonly accounted mechanism of injury leading to lower limb fracture in both clinical and forensic practice. The direction of force application required to fracture the long bones of the leg via three-point bending has traditionally been interpreted using the Messerer or wedge fracture. As bones are weaker in tension than in compression, the side of the bone in tension, opposite the impacting force, fractures first. The fracture propagates across the bone along two lines of cleavage to create a wedge. Early opinion suggested that the broad base of this wedge occurred on the side of impact. However, multiple studies have cast doubt on this theory, reflecting that in “real life” the fracture pattern is not predictable. In recent years, researchers have sought to improve the accuracy of bone trauma interpretation. In 2018, Christensen et al. applied fractography techniques to long bones; a team of materials scientists and forensic anthropologists were able to successfully determine the direction of fracture propagation by assessing the fracture surface in human femora.<sup>1</sup> A limited number of case studies suggest that volume-rendered reconstructions derived from CT scans can demonstrate fractography features, but to date there is no published comparison of CT and direct bone visualization.<sup>2,3</sup>

**Method:** Fifteen porcine femora were broken using a three-point bending test with axial loading to simulate external trauma, creating 30 fractured bone samples. These were scanned using CT and macerated. The CT images were analyzed for the presence or absence of five fractography features described by Cristensen et al. (bone mirror, bone hackle, wake features, arrest ridges, and cantilever curl) by a radiologist and findings compared to forensic examination of the bone by an anthropologist. Both assessors were asked to comment on the side of impact.

**Results:** Porcine bones demonstrated fractography features on CT. The radiologist and the anthropologist showed complete agreement for the presence of “arrest ridges” caused by compressive force. Although there was no significant agreement as to the presence or absence of the other four fractography features, both assessors correctly inferred the side of impact for all specimens.

**Conclusion:** Postmortem CT is a recognized and well-regarded technique in the investigation of fatal trauma and has been used as both an adjunct and an alternative to autopsy. CT fractography demonstrates arrest ridges on fractured bone surfaces, and, therefore, the detection of this feature could be used to determine the side of force impact, for example, in lower limb trauma in road traffic collisions. This novel technique may facilitate a more accurate and non-invasive interpretation of fracture patterns and add value to standard imaging techniques.

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1. Christensen A.M., Hefner J.T., Smith M.A., Blakely Web J., Bottrell M.C., Fenton T.W. Forensic Fractography of Bone: A new approach to skeletal trauma analysis. *Forensic anthropology*. 2018; 1: 32-51.
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### Computed Tomography, Blunt Force Trauma, Fractography

## H141 The Evaluation of the Routine Use of Postmortem Computed Tomography (PMCT) In a High-Volume United States Medical Examiner's Office

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**Learning Overview:** The goal of this presentation is to provide data regarding the effectiveness of integrating PMCT into routine death investigation in a high-volume United States medical examiner's office.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the degree to which PMCT imaging findings were appropriately identified and incorporated into the death investigation report and the cause and manner of death through a retrospective radiology audit of 10% of cases over a nine-month period.

This study used a high-volume United States medical examiner's office that routinely performs pathologist-interpreted PMCT on nearly all cases brought in for autopsy or external exam to determine whether significant imaging findings were appropriately identified and incorporated into the death investigation report and the cause and manner of death. A retrospective audit of 10% of cases receiving PMCT over a nine-month period was performed.

A total of 200 cases from four categories were reviewed: (1) autopsy without a Radiology Consult (RC) ( $n=77$ ); (2) external exam or partial autopsy without RC ( $n=79$ ); (3) autopsy with RC ( $n=26$ ); and (4) external exam with RC ( $n=18$ ). Due to the limited number of RC cases, all available RC cases were included, while categories 1 and 2 were sampled randomly.

A forensic radiologist with ten years' experience (who did not consult previously on the cases) reported the PMCT findings. The radiologist and a pathologist (not the case pathologist) reviewed the death investigation report in tandem to document any PMCT findings omitted from the report. Omitted findings were categorized into seven error types according to a modified Goldman classification: Major 1—Unrecognized fatal injury or pathology that would change Cause of Death (COD) and/or Manner of Death (MOD). Major 2—Unrecognized fatal injury or pathology that would not change COD and/or MOD. Minor 3.1—Incidental finding unrelated to COD, but potentially important (e.g., for public health). Minor 3.2—Additional finding related to the mechanism of a recognized COD. Minor 4.1—Incidental finding unrelated to COD and not important. Minor 4.2—Additional findings related to recognized COD but not important. Minor 5—Anatomic error (e.g., rib number). Errors that were difficult to classify were reviewed by four people to reach a consensus.

A total of 13 Major errors (four Major 1 and nine Major 2) were identified (6.5%). In all cases where Major errors were identified, the MOD was certified as either accident or natural. The four Major 1 errors were found in cases that received external examinations without RC. Of nine Major 2 errors, two occurred in cases receiving full autopsies without RC, and six occurred in cases receiving external examinations without RC. Only one Major error was found in an RC case (Major 2, external examination). The data is summarized in the table below. In only one case did the Major error suggest a possible change in the MOD (a missed humerus fracture in a decomposed, non-suspicious death certified as natural). In none of the 200 cases did a missed PMCT finding definitively change the MOD.

Three of four Major 1 and two of nine Major 2 errors in external examinations were fatal abdominal pathologies (e.g., pancreatitis). Six of nine Major 2 errors, including one RC case, were fatal injuries (e.g., cervical spine injury) missed in cases with other multiple fatal injuries. The RC was requested specifically for evaluation of a fetus found in a female motor vehicle accident victim.

Minor unrecognized (or undocumented) PMCT findings were extremely common, occurring in 95% of cases overall. Little difference was observed in minor errors frequency between the four case categories. Of the minor error types, Minor 4.1 (unrelated to COD and not important) was the dominant type, occurring in 83.5% of cases.

In conclusion, pathologists reliably interpret PMCT scans, avoid major errors, and accurately certify deaths in the vast majority of cases. As major errors occur more frequently in external examinations (which are primarily naturals and accidents), some educational interventions, such as increased training for pathologists in the radiological appearance of abdominal pathologies and trauma, should be considered. The data suggest that RCs may reduce major errors, although RC efficacy cannot be determined from this study due to the small number of these cases. Here is a summary of results:

Modified Goldman Classification	Total (%) $n=200$	Autopsy (%) $n=77$	External or Partial Autopsy (%) $n=79$	Autopsy with RC (%) $n=26$	External with RC (%) $n=18$
<b>Major (total)</b>	<b>13 (6.5%)</b>	<b>2 (2.6%)</b>	<b>10 (12.7%)</b>	<b>0 (0%)</b>	<b>1 (5.6%)</b>
Major 1	4 (2%)	0 (0%)	4 (2%)	0 (0%)	0 (0%)
Major 2	9 (4.5%)	2 (2.6%)	6 (8.3%)	0 (0%)	1 (5.6%)
<b>Minor (total)</b>	<b>191 (95.5%)</b>	<b>72 (93.5%)</b>	<b>78 (98.7%)</b>	<b>23 (88.5%)</b>	<b>18 (100%)</b>
Minor 3-1	25 (12.5%)	5 (6.5%)	10 (13.9%)	5 (19.2%)	3 (16.7%)
Minor 3-2	34 (17%)	12 (15.6%)	18 (22.8%)	2 (7.7%)	2 (11.1%)
Minor 4-1	167 (83.5%)	64 (83.1%)	66 (83.5%)	22 (84.6%)	15 (83.3%)
Minor 4-2	136 (68%)	41 (53.2%)	68 (86.1%)	12 (46.2%)	15 (83.3%)
Minor 5	19 (9.5%)	7 (9.1%)	19 (9.5%)	4 (15.4%)	0 (0%)

### Forensic Pathology, Postmortem Computed Tomography, Death Certification

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## H142 Cellular FLICE-Like Inhibitory Protein (C-FLIP) and Troponin-I: Promising Markers for the Determination of the Vitality in Suicidal Hangings

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**Learning Overview:** The goal of this presentation is the investigation of the potential use of C-FLIP (an intracellular protein involved in receptor-induced apoptosis) and Troponin I (fsTnI, fast skeletal muscle) in forensic practice to perform differential diagnosis between suicidal and simulated hangings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by opening promising new horizons in forensic pathology, hopefully leading to further studies comparing these new molecules to classical immunohistochemical markers of vitality.

Differentiating between suicidal hanging and simulated hanging (suspension of the victim after murder) represents one of the most challenging problems for forensic pathologists. Beyond macroscopic findings, a diagnosis of suicidal hanging can only be carried out by investigating vitality signs of the ligature mark and underlying soft tissues. However, conventional macroscopic and histological findings may be unreliable; a great help in the determination of ligature marks' vitality can be provided by immunohistochemical methods. To date, immunohistochemical studies have shown good reliability with anti-myoglobin and anti-fibrinogen antibodies, as well as Anti-P-Selectin and anti-tryptase antibodies.

The expression of C-FLIP antibody and Troponin I, fast skeletal muscle (fsTnI) antibody in samples obtained from 21 subjects who died of hanging were examined in order to evaluate their potential use in forensic practice. C-FLIP acts as an apoptotic inhibitor, and its hyperexpression has proven to be related to human neoplasms. In ligation marks, compression-induced ischemia determines, via tissue hypoxia, under-regulation of C-FLIP and activation of caspase-8-related apoptotic paths. Troponin I, a muscle fast fiber-specific marker of skeletal muscle injury, is linked to the fact that the fast fibers have a lower concentration of ATP and, consequently, are more susceptible to ischemic damage due to constriction exerted by the noose.

Cases included 8 women and 13 men, mean age of 52.2 years, who died from suicidal hanging. The control group included six women and four men, mean age of 47.3 years, who died from opioid overdose ( $n = 2$ ), car accident ( $n = 3$ ) and sudden cardiac death ( $n = 5$ ).

The skin samples of the neck for C-FLIP testing were taken diametrically opposite to the suspension point (where the maximum load occurs). As positive control for Anti-FLIP antibody (ab8421), neoplastic prostate and healthy kidney samples were selected. To perform negative controls, primary antibody was omitted and replaced with Phosphate-Buffered Saline (PBS). Slides were counter-stained with hematoxylin.

The analysis on fsTnI was carried out on sternocleidomastoid and infrahyoid muscles samples, at the same level as the skin samples. Positive testing for Anti-Troponin I antibody was carried out on a case of myocardial infarction.

All samples, after fixation in formalin, dehydration, and embedding in paraffin, were stained on an automated immunostainer, using polyclonal antibodies.

In all cases (21 out of 21) who died by hanging, a clear and evident intracytoplasmic depletion of C-FLIP was appreciated. No substantial differences were found in relation to type of hanging (complete or incomplete), knot position, or noose material. Only 19 out of 21 cases showed clear and evident intracytoplasmic depletion of Troponin I. The remaining cases showed lack of intensity reduction; this could either be due to noose material or to the fact that death occurred due to cardiac arrest, triggered by an inhibitory stimulation of neck's neurovascular bundles.

Results of this study suggest the possibility of discriminating between antemortem and postmortem hangings, especially when C-FLIP and fsTnI are tested in combination. The present study opens new possibilities in forensic pathology, hopefully leading to further studies comparing these new molecules to classical immunohistochemical markers of vitality.

### Immunohistochemistry, Vitality, Hanging



## H143 Suicidal Firearms Injuries of the Back of the Head/Neck: A Case Series

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**Learning Overview:** After attending this presentation, attendees will understand that, although rare, suicidal gunshot wounds of the back of the head do occur, and they can involve handguns as well as long guns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting four cases of suicide by firearm wherein the decedent shot himself in the back of the head or neck.

Suicide by firearm is relatively common in certain parts of the world. Depending on firearm availability, any gun type may be used for suicide, including handguns, shotguns, and rifles. Common entrance wound sites in suicidal gunshot wounds include the head and chest, with classic head wound locations being the temple, the forehead, the submental chin, and intraoral. Only occasional suicidal gunshot wounds occur in atypical body locations or involve unusual situations. In this report, four cases of suicidal gunshot wounds of the back of the head/neck are presented.

**Case 1:** A 57-year-old male was on short-term disability from work due to recently diagnosed lupus, with associated encephalopathy, seizures, and severe behavioral changes, including outbursts of anger. He also had severe depression to such an extent that family members had reportedly locked up all of his firearms. After a verbal outburst of anger toward his wife, he entered his basement, then his wife heard a gunshot. A .38 caliber revolver was found near his body. He was emergently transported to the hospital, where he was eventually pronounced dead. At autopsy, there was a tangential, medium-range gunshot wound of his right posterior parietal scalp, with an underlying keyhole defect of the bone, and projectile fragments recovered from within the subcutaneous tissues. The cause of death was a gunshot wound of the head. The manner of death was suicide.

**Case 2:** A 22-year-old male was found dead in his bathtub on a welfare check after he had not arrived to work for two days. He had texted his father earlier that day, telling him that he loved him and to keep moving forward no matter how grim life might be. He was nude, and there was a recently purchased 9mm semi-automatic handgun in the tub with him. At autopsy, a contact-range gunshot entrance wound was on the posterior midline of the head, with an exit wound of the central lower forehead. According to family members, he was socially awkward and struggled with “sexual identification issues.” The cause of death was a contact gunshot wound of the head. The manner of death was suicide.

**Case 3:** A 50-year-old man was found dead at the edge of some woods, in a field near the rural home that he shared with his parents. He had a history of chronic back pain, depression, anxiety, inability to find employment, as well as financial stress, and had lived with his parents for the past ten years after having been employed as a registered nurse and being arrested and imprisoned on drug-related charges. His father found his body after the father discovered a suicide note in the residence, went outside to look for his son, then heard a gunshot. His body was found on snow-covered ground, without snow-track evidence of anyone else nearby. A double-barrel, 20-gauge shotgun was found near the decedent. Autopsy revealed a contact shotgun entrance wound of the posterior midline neck, with a gaping exit wound of the left side of the face. Measurements of the gun’s trigger-to-barrel and the decedent’s entrance wound-to-fingertips indicated that the man could have easily fired the shot. The cause of death was a shotgun wound of the neck/head. The manner of death was suicide.

**Case 4:** A 26-year-old man was found dead by police in his secured apartment, after the man’s employer requested a welfare check. The decedent had called off from work, stating that he was “mentally unable to work.” This statement raised concerns for his boss, as he knew that the man struggled with depression and suicidal thoughts. His body was found prone, on the floor, with his head turned toward the right, and his 9mm handgun clasped within his left hand, near the back of his head. At autopsy, a contact gunshot entrance wound was present on the midline occipital scalp. An exit wound was on the central right forehead, near the nasal bridge. The cause of death was a gunshot wound of the head. The manner of death was suicide.

Each case will be presented, with special attention given to investigative and autopsy findings which indicated suicide as the manner of death.

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### Forensic, Gunshot Wound, Posterior Head

## H144 Roderick Covlin's Botched Bathtub-Staged Homicide: Aquatic Investigative Techniques to Get It Right From the Start

*Andrea Zaferes, BA\*, AZ Training/Lifeguard Systems, Shokan, NY 12481*

**Learning Overview:** After attending this presentation, attendees will have a novel approach to investigating staged bathtub homicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by applying effective aquatic crime scene investigation procedures to body-reportedly-found-in-bathtub cases.

Roderick Covlin was an unemployed, lavish, womanizer, failed stockbroker, Taekwondo expert, and scheming professional backgammon player, engaged in a bitter divorce from his estranged, wealthy, banking executive wife. On New Year's Eve morning, 2009, Shele *Danishefsky* was found dead in her bloody bathtub by their 9-year-old daughter. The family declined an autopsy for Jewish Orthodox reasons and because the notable death appeared as an accidental slip and fall resulting in drowning. Covlin was not examined for injuries, the staged drowning scene was not processed, and evidence was not secured from the New York City luxury apartment because of the accident assumption.

Later, family concerns brought about an exhumation autopsy on March 1, 2010, resulting in a homicide due to "neck compression" diagnosis. Only then was an aquatic investigation applied, which raised factual suspicions.

Covlin then attempted to access the 5.2 million dollar inheritance by framing his daughter with a fake confession note, trying to get her to physically stage a rape by her custodial paternal grandfather, and spoke of plans to marry her off in Mexico at age 14.<sup>1</sup>

A staged crime scene entails a deliberate attempt to misdirect the investigation by altering the scene and events to disguise what actually occurred.<sup>2-4</sup> Staging can include directing investigators to incorrect perpetrators, disguising homicides as missing person cases, and making homicides resemble accident, suicide, or natural deaths.<sup>5,6</sup> Homicides staged as water-related or bathtub deaths are often misdiagnosed as accidents or suicides.<sup>7,8</sup> Aquatic scenes, like crash and fire scenes, require specialized investigative techniques.

Multiple indicators of possible deception existed prior to the autopsy that likely would have been caught if aquatic investigative techniques had been initially performed. Covlin disguised his land-based asphyxiation (strangulation) as an accidental aquatic asphyxiation (drowning) by posing *Danishefsky's* naked corpse in a bathtub full of water.<sup>9</sup> Ergo, a bathroom aquatic crime scene.

Diligent aquatic investigative techniques were required to recognize, decipher, and document scene inconsistencies, questionable events, dubious circumstances, physical attributes and conditions of reporting parties, and specious party statements. An evidence-based "Bathtub/Bathing Environment Investigation Form" was used addressing: (1) if it made sense that the decedent was in that water at that time; (2) if the location, position, posture, physical state, and scene of the decedent and reporting party(s) made sense; and (3) sensical reasoning for the decedent not surviving submersion. The questionnaire included detailed information from first responders, law enforcement, scene information, information about reporting parties, and the decedent's body, bathing habits, victimology, and medical history.

This audacious case demonstrates the necessity of competent aquatic incident procedures to reconstruct the altered scene and event. The methodical assessment employed probing aquatic investigative principles, regimented techniques, substantive pre- and postmortem investigative recommendations, and reconstructing reporting party statements and behaviors to test their veracity.

Covlin's fallacious statements and the anomalies contradicted the physical evidence, and the crime scene. For example: (1) signs of struggle initially assumed to be a slip-and-fall; (2) *Danishefsky* bathing with her hair down after a same-day, expensive, "perm" (hairstyle) treatment; (3) victim's injuries (anterior, posterior, and lateral, superficial, external contusions, abrasions, and a laceration); (4) bathtub water levels and displacement; (5) reporting party observation lines of sight and statements; (6) decedent's position and state on bathroom floor as observed by Emergency Medical Services (EMS) and documented by investigators versus decedent's purported positions in the bathtub when found by daughter and Covlin; (7) water flow sounds reported by daughter at night; (8) forensic pathology neck findings (hemorrhages and hyoid bone fracture); (9) martial arts choke hold statements; (10) 911 telephone statement; (11) water flow rates; (12) medical evidence; (13) victimology; (14) Covlin's inappropriate behavior before, during and after the crime (e.g., maliciousness, lack of panic, grief and sorrow); and (15) lack of accidental cause for incapacitation leading to drowning.

The botched Covlin/*Danishefsky* crime scene was a conscious effort to thwart its investigation by a conniving perpetrator. On March 11, 2019, reality trumped fiction. Truth and justice prevailed. Covlin was found guilty of second-degree murder and received the maximum sentence of 25 years to life.

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  7. Water-related murders were undetected until a subsequent homicide was recognized as in the cases of Drew Peterson, Timothy Boczkowski, Coral Watts, and George Smith.
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### **Staged Crime Scene, Homicide, Asphyxiation**



## H145 Clinical Forensic Medicine at the Georgia Bureau of Investigation Medical Examiner Office

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**Learning Overview:** The goals of this presentation are to discuss the history, services, and statistics of the Georgia Bureau of Investigation (GBI) clinical forensic medicine program and discuss example case details and dispositions of living victim consultations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by how a clinical forensic medicine program at a medical examiner's office effectively applies forensic science to determine etiology of injuries and assist in legal solutions concerning living victims.

In fatal cases of child abuse and neglect, the office of the medical examiner is responsible for death investigation and autopsy examination to determine the cause and manner of death. Medical examiners are rarely involved in evaluating and interpreting injuries in living victims of trauma. Given their experience and training, they are uniquely qualified to assimilate historical, social, and medical information to accurately assess non-fatal injuries and assist in the investigation of child abuse and neglect.

The GBI Medical Examiner Office (MEO) established a clinical forensic medicine program in August 2000—the Child Abuse Investigative Support Center (CAISC). Recent data reports over 82,000 investigations for child abuse and neglect in Georgia. CAISC provides expert consultation and educational child abuse training to address the needs of agencies in Georgia involved in investigations of child maltreatment.

This study is a review of CAISC providing 18 years of clinical forensic medicine service. The review provides details of the living victim consultations, including age, gender, requesting agency, spectrum of injuries, and opinion of injury causation. Additionally, the data of the educational training is reviewed.

This study updates a previous review of CAISC in 2011, which was prior to the establishment of Children's Healthcare of Atlanta (CHOA) Stephanie V. Blank Center for Safe and Healthy Children. This center is physically based in metropolitan Atlanta, GA, and has a team of board-certified child abuse physicians who conduct evaluations of victims of suspected child abuse and neglect. Child abuse pediatrics has emerged as the clinical specialty that assumes responsibility for assessing injuries in non-fatal suspected child abuse. With this review, CAISC was identified as a valuable resource to: (1) serve agencies in areas of the state that lack the resources and medical expertise that are available in metropolitan areas; (2) provide expertise when comprehensive case information is not available in the acute setting of injury; (3) provide a second opinion in questionable or contentious cases; and (4) provide training opportunities to support state agencies responsible for recognizing and reporting suspicious injuries.

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### Clinical Forensic Medicine, Living Victim, Child Abuse



## **H146 Forensic Medicolegal System in Qatar**

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**WITHDRAWN**



## I1 Psychological Trauma in South Carolina Asylum Seekers

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**Learning Overview:** After attending this presentation, attendees will better understand the rates and types of trauma incurred in asylum-seekers and other legal protection-seeking migrants in the American South.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the empirical literature on non-citizen migrants in the American South. Given the scarcity of resources available to clinicians and other advocates for this population, this presentation will improve clinicians' ability to diagnose and treat trauma in migrant populations. Additionally, this project aims to better understand why the local asylum-seeking population may face such an unusually low asylum grant rate.

The American South is home to a significant but understudied population of non-citizen migrants undergoing court proceedings to be considered for asylum status.<sup>1</sup> In the United States, asylum seekers may apply for legal protection due to a past history or current threat of persecution based on their race, religion, nationality, social group, or political opinion.<sup>2</sup> As of 2019, the United States was the largest recipient of new asylum applications in the world. Nearly half of the 301,000 asylum applications that the United States received in 2019 originated from Central America and Venezuela.<sup>3</sup> In addition to the asylum process, many migrants already in the United States may alternatively apply for legal protection under the Violence for Women Act (VAWA) or U-visa (i.e., United States non-immigrant visa for crime victimization occurring in the United States).<sup>4,5</sup>

Although South Carolina (SC) has over 5,000 individuals seeking asylum or legal protection, it possesses one of the most challenging environments in which to seek legal protection. Since the state itself does not have an immigration court, migrants living in SC must travel to the nearest immigration court in Charlotte, NC. However, the Charlotte immigration court possesses one of the lowest asylum grant rates in the country. In 2018, Charlotte approved only 6.1% of asylum cases whereas the national approval rate was at 29.4%.<sup>6</sup> This population of legal protection-seeking migrants in SC is unique due to the unsupportive political climate in the region and the fact that it has not been the subject of empirical study.

Utilizing data from the Medical University of South Carolina (MUSC) Asylum Clinic, this project aims to assess the demographics of asylum-seekers, U-visa, and VAWA applicants, the types of trauma they have endured, and their mental health disorder diagnoses. Specifically, this presentation will discuss the rates of post-traumatic stress disorder, major depressive disorder, generalized anxiety disorder, and other stressor-related disorders present in this population. Data obtained from the clients assessed at the clinic (current  $N=29$ , though this is an ongoing data collection project) will be compared to other similar migrant populations nationally and worldwide.

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### Asylum, Trauma, Immigration Court

## I2 A Proposal for a Scientific Forensic Model Applied to Missing Persons Searches

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**Learning Overview:** The goal of this presentation is the proposal of a model to search for missing persons using forensic science methodologies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the effectiveness of research by increasing the chances of finding missing persons in a short time while they are still alive.

The ability of the scientific community to increase the effectiveness of research will improve the chances of finding missing persons both alive and in a shorter time frame.

In Italy, from January 1, 1974, to June 30, 2020, there were 250,008 missing person reports; 188,182 people were found, but 61,826 are still missing. Of those reported missing, 51.71% are of Italian nationality and 48.29% are foreigners. Just within the first half of 2020, there were 4,833 missing person reports; 3,052 people were found and 1,781 people are still missing.

Cell phones, social networks, and even car keys with transponders are all systems that allow a precise geolocation of a person. There are also applications that track cell phones that are off or without a signal, using only three words (such as [www.what3words.com](http://www.what3words.com)), through geographic profiling (or predictive spatial analysis), or, finally, through the analysis of satellite images that scan daily and allow “the invisible” to be seen through multispectral bands. Parallely, another tool that was created for other purposes is under experimentation in Italy; it is the “psychological profile” reconstruction of the missing person. This technique uses the psychological mechanism of the mind focused through mnemotechnics and cognitive interview; obtaining much useful research information and details from family members and people who are significant to the person.

In particular, it is very useful to have information about the places beloved by the missing person, those she/he has frequented, and those she/he would have liked to go to. There is also a chance that this could be derived from shared memories that can link that person to those places. It is useful to know the person’s social network and where these contacts are located.

If it were possible to have a joint effort from both the psychological and geographic teams in a timely manner, it would be possible to understand and identify through a predictive analysis (such as that of the film *Minority Report* with Tom Cruise) the area where the person disappeared.

Some case studies followed by a Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis will better explain the application of this model.

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### Forensic Model, Missing Persons, Searches

## I3 Mental Health Crisis Response Teams and the Challenges of Evidence-Based Police Reform

Dana M.C. Valdez, MD\*, Los Angeles, CA 90026

**Learning Overview:** After attending this presentation, attendees will: (1) have an increased understanding of the role of law enforcement in mental health emergencies, (2) learn the history of mental health crisis response teams, (3) learn how they are typically implemented, and (4) the data behind them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a review of mental health crisis response teams, the evidence supporting them, and areas where further research would be helpful to inform public policy decisions.

It is not unusual for People with Mental Illness (PMI) to interact with law enforcement. In the United States, 25% of PMI have been arrested at some point in their lives, and between 6% and 10% of police contacts with the public involve a person with serious mental illness.<sup>1,2</sup> Beginning with de-institutionalization in the 1960s, the burden of assisting PMI in crisis has fallen increasingly on law enforcement.<sup>3</sup> Police officers are typically the first responders if a PMI has allegedly violated the law, appears to be acting bizarrely, or is otherwise unable to care for themselves. They act as triage workers, determining whether a PMI should be taken for emergent mental health evaluation, arrested, or linked with local support services such as community mental health clinics and homeless shelters. To address this evolving role, police departments in the 1980s widely began to implement training programs for their officers regarding interactions with PMI; however, this alone has not proven to be sufficient.<sup>4</sup>

Mental health crisis response teams have been broadly implemented in cities across the country to help meet this need. They arose out of the 1987 police shooting death of a Memphis man with mental illness and were created for the purpose of decreasing officer and civilian harm during psychiatric crises. These situations have been found to end in violence more frequently—a study on Medicaid® data found that individuals with schizophrenia are three times more likely to die from encounters with law enforcement officials than the general population.<sup>5</sup> There have been a variety of crisis response team models implemented in the United States, typically in two broad forms: police-based (e.g., officers with special training or with embedded mental health workers) and mental-health based (e.g., field-based mental health evaluation teams).

Although crisis response teams have existed in various implementations since 1987, data regarding their efficacy are both limited and unclear. Available data regarding the efficacy of these models in terms of decreasing arrests, officer/civilian injury, or increasing mental health linkage are mixed.<sup>6</sup> Other studies note that PMI often report difficulty with mental health follow-up and case management after their initial encounter with first responders.<sup>7</sup> This lack of data is important from a public policy perspective, especially in the wake of increased scrutiny of police and the budgets of law enforcement agencies in 2020. Media reports and public discourse have called attention to the lack of community mental health resources and increasingly point at initiatives like crisis response teams as a “safer” approach to police involvement with PMI.<sup>8</sup> Municipal officials are beginning to heed similar calls—in July 2020, the Los Angeles County Board of Supervisors announced plans to close one of its largest jails in favor of diverting funds for mental health treatment and other programs.<sup>9</sup>

In order to make public policy decisions like this, officials should have access to accurate data about the efficacy of interventions, including crisis response teams. This presentation will summarize existing data on crisis response teams and highlight areas where further research is needed to help inform these choices. Existing systemic barriers faced by crisis response teams in accomplishing their goals, such as increasingly long wait times for inpatient psychiatric admission, will also be discussed.

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### Police, Mental Health, Public Policy



## I4 Testifying in Court in a Forensic Mental Health Case: Opinions From the Medical and Legal Sides of the Room

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**Learning Overview:** After attending this presentation, attendees will be aware for the difficulties that both forensic mental health experts and the legal professionals encounter in a court case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need to increase awareness of the concepts of forensic mental health in court and by limiting biases.

In the interface between the Science and the Law, the forensic mental health expert's role is to provide evidence that guides legal decision-making. Experts must base their forensic reports and subsequently their testimonies on scientific and medical findings. Additionally, the experts must ensure that fact finders (i.e., judges, jurors, and lawyers), who may have no background knowledge on the topic, understand the concepts used and their conclusions. To make matters more difficult, experts must provide their expertise in constrained, time-limited settings, where they are not the ones leading the conversation. In some health systems, forensic experts also have a dual-role conflict whereby they are both providing care and treatment for the individual being prosecuted and at the same time are providing testimony that can defend or prosecute them. The clinician's natural tendency to empathize and be supportive toward the client can cloud judgment; thus, these experts must always be aware of their biases and focus on objectivity.

Forensic experts thus have an enormous role and an enormous impact on the lives of those being tried by the justice system. Recent high-profile cases across North America have made this clear. Testimonies and reports made by mental health experts can often have ramifications on public perceptions of the criminal justice system and play a role in mental health stigma. All of this brings to light an important question—are forensic psychiatrists and psychologists effective at their task? In other words, are forensic mental health experts well-suited and well-trained to provide the same level of scientific knowledge that other forensic areas provide? Furthermore, is the scientific knowledge in psychiatry and psychology sufficient enough to be effectively utilized by legal professionals and jurors? These questions are important and poorly understood, as evidenced by the diverse opinions that psychiatrists and psychologists can provide within the same case and the difficulty that jurors can have at understanding the complex medical concepts.

The aim of this presentation is therefore to provide a literature review on multiple aspects of the role of forensic mental health experts in court. The review will explore the perceptions of these experts in court; particularly through the lens of the experts themselves and also the legal professionals and jurors. This presentation will notably consider the biases and limitations on both sides and highlight some recommendations for improving the practice for mental health professionals. Additionally, this presentation will explore the training that forensic mental health experts receive and comment on their suitability and effectiveness in equipping experts with the tools to perform this role.

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### Psychiatrist, Testimony, Bias

## I5 The Forgotten Ones: Post-Traumatic Stress Disorder (PTSD) in First Responders

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**Learning Overview:** The goal of this presentation is to discuss: (1) various experiences of first responders from Emergency Medical Services (EMS), police, crime scene investigators, physicians, and others; (2) different agency and regional responses to PTSD and processing of PTSD and grief; and (3) symptoms experienced in these groups. How chronic exposure to trauma and vicarious trauma affect responders' mental health will also be identified.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a serious aspect of mental health and responder well being by discussing vicarious trauma, ways to recognize symptoms, and resources available for treatment. Understanding how PTSD is affecting first responders may also help address the need to research the forensic science community's potential risk. The forensic science community is not immune to the effects of PTSD and should also investigate how PTSD could be affecting the members of the forensic science community.

This presentation will discuss how PTSD can affect first responders in federal, state, and local agencies such as local fire departments, law enforcement, emergency medical services, and public health officials. In this presentation, current research studies and data will be presented to help identify how PTSD is affecting first responders who are continuously exposed to traumatic and stressful incidents over the course of their careers.

PTSD and acute stress reactions are not reserved for crime victims and war veterans. The American Psychiatric Association defined PTSD during the 1980s in the 3<sup>rd</sup> edition of *Diagnostic and Statistical Manual of Mental Disorders*.<sup>1</sup> Researchers have extensively researched the effects of PTSD on military veterans returning from conflict whereby mental health professionals were then able to use standard criteria to properly diagnose patients who had PTSD. Since the 1980s, researchers are branching out from focusing on military veterans who have PTSD to researching the effects of PTSD on first responders.

The nation's first responders experience a wide variety of mental health symptoms that often go unrecognized. Recent research suggests that first responders such as firefighters, emergency medical technicians, paramedics, police officers, crime scene investigators, and allied health professionals are just as vulnerable to the effects PTSD. This presentation's focus will address the history of PTSD, recently published research, and new research areas to be studied in the future. For example, according to Stanley, the research data uncovered some shocking results of 1,027 retired and current firefighters that found that 46.8% had suicidal ideations.<sup>2</sup> First responders have been overlooked over the years without the opportunity to ask for help. This can be attributed to the culture and stigmatism of being considered weak if one asked for mental health help to overcome the traumatic stress encountered on the job. Many first responders have suffered in silence, and the only way first responders will be able to get help is through further research into how PTSD affects first responders and the public acknowledgment that PTSD is a real problem destroying first responder's lives.

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### PTSD, Vicarious Trauma, PTSD-C

## I6 Deviant Behavior in Relationships Between Humans and Animals

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**Learning Overview:** After attending this presentation, attendees will have a clearer understanding of the application of a predictive model of criminal behavior (profiling) that is based on the relationship between the perpetrated abuse of animals by some individuals and the possibility that such deviant acts may represent precise symptoms in order to predict and contain future aggressive actions toward individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making it clear that there is a method by which attendees can identify certain deviant symptoms in the actions of adults or minors who torture and kill animals by studying appropriate parameters to be entered in a database aimed at preventing murders and identifying the offenders.

This study is based on the understanding of the following parameters: (1) paraphilias and deviances related to the dominion on animals such as: zooanthropology, zoosadism, and zooerastia; (2) the LINK evaluation of the act that the offender perpetrates against the animal; and (3) the study of serial cases of murder and torture. The zooanthropology of deviance studies the relationships between man, animal, and animal cruelty.

Since the advent of agriculture in 10,000 BC, man has domesticated animals by being in a dominant position with respect to them. Some paraphilias, characterized by sexual and predatory nature are relevant behavioral pathologies based on the enjoyment in inflicting pain on what one possesses. For the offender, the animal becomes an object. LINK is a criminal concept and indicates the correlation between animal cruelty and the killing of animals. It favors the possibility of preventively intercepting the worsening of criminal behavior. Even in non-pathological subjects, from a psychiatric point of view, LINK reveals the social dangerousness of these individuals and the need to raise the sentences for animal-related crimes (in Italy, they are a myth). The Federal Bureau of Investigation (FBI) defines animal cruelty and in 2016 the Behavioral Analysis Unit (BAU) added animal cruelty in the Uniform Crime Reporting (UCR). The UCR is used nationwide in investigating crimes such as domestic violence, stalking, rape, and even serial murders. Criminal studies show that detecting minors who have tortured animals and intervening helps to prevent future deviant behavior. Finally, this presentation suggests an extensive database at the European level, based on the transposition of the parameters set out in this work. It would use the data on crimes against animals, putting offenders in the system with criminals and create a profile that could be used to prevent and track down those who commit criminal homicide.

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### Criminal Homicide, Animal Cruelty, Profiling



## **I7 The Relationship of Firearm Possession to Attachment Styles and Psychological Well-Being**

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**WITHDRAWN**

## I8 Trends in Suicide: A Postmortem Analysis of a 20-Year Case History by the Unit of Legal Medicine of Pavia

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**Learning Overview:** After attending this presentation, attendees will recognize the importance of more deeply studying the phenomenon of suicide from several points of view, especially including the retrospective and the postmortem views, for the immense value these data have in the prevention field.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by recognizing trends about suicide phenomenon and providing knowledge in order to perform tailored programs to this population, offering evidence-based treatment and gender-sensitive methods to these vulnerable individuals.

In 2012, as reported by the World Health Organization (WHO), 804,000 suicides have been documented worldwide with an annual standardized global rate of suicide of 11.4 individuals per 100,000 population.<sup>1</sup> Italy ranks among the countries with a low suicide rate; in 2015, there were 6.1 per 100,000 who died from suicide. Even though it is a lower value than the European trend, suicides still remain a public health problem. Analyzing the phenomenon in detail, important aspects emerge regarding the characteristics of the suicide victims.

**Aims:** The aim of the study is to investigate the phenomenon of suicide in cases analyzed by the Unit of Legal Medicine and Forensic Sciences “A. Fornari” of Pavia, in the 20-year period from 1999 to 2019. The evaluation is not limited to providing useful qualitative-statistical data, but represents an important starting point to further investigate the risk factors related with suicidal events.

**Materials and Methods:** For each case, the following variables were taken into consideration: personal data, social status, medical, and forensic evaluations.

**Discussion:** In the 20-year period examined, 724 autopsies involving subjects whose death had been classified as suicide were performed (545 men and 179 women). The average age of the cases examined was  $50.16 \pm 20.2$  years (12–100 years). The most frequent cause of death was mechanical asphyxia concerning the death of 334 people, followed by self-inflicted gunshot wound (117 deaths), poisoning (86 deaths), falls from height (81 deaths), road traffic injuries (39 deaths), stab or cut wounds (35 deaths), and death caused by fire (17 deaths). Regarding the most frequent causes of death analyzed by gender, the data shows that the most frequent method in both genders is mechanical asphyxia. It is interesting to highlight that in males the most frequently chosen method is hanging (drowning/other acute mechanical asphyxias ratio of 1:6), while drowning is more frequent in women (ratio of 1:1). In a postmortem analysis, assessing premeditation is rather complex; nevertheless, it has been reported that 58 (9%) subjects wrote a “suicide note” before dying. The average age of these subjects is lower than of the whole group. These data correspond to the known fact that premeditation is more distinctive of adulthood, while impulsivity is more typical of adolescents and young adult subjects. Marital status has been studied as well: the “never married” people (34%) were on average younger than the subjects of the entire case history ( $39 \pm 17$  years). The separated or divorced subjects were also relatively younger ( $49 \pm 15$  years). The correlation between employment and cause of death has been studied. A higher trend to use drugs to commit suicide has been observed in the “helping profession” and to use gunshot wounds in policemen and security guards. Surprisingly, this study detected a much lower percentage of mental illness than what is expressed in the literature. The subjects who suffered from depression are 189 (26%), alcohol abuse was reported in 30 individuals (4%), drug addiction in 23 subjects (3%), and psychosis in 21 subjects (~3%). It is important to point out, however, how these data could be potentially underestimated considering the difficulty of obtaining a certain diagnosis in the psychiatric field.

**Conclusion:** Although the above-stated aspects are clearly known in the literature, it is essential to continue monitoring them in their various realities, for the purpose of intervening with prevention campaigns. Forensic medicine, indeed, represents an essential discipline, not only for its legal implications, but also for its social and ethical connotations.

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### Suicide, Retrospective Study, Manner of Death



## I9 Borderline Personality Disorder and Religion: An Unusual Marital Relationship

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**Learning Overview:** After attending this presentation, attendees will better understand that religion or spirituality can influence the severity of psychopathology. Some studies suggest that strong religious beliefs can be found in people who experience more severe psychiatric symptoms.<sup>1</sup> In this context, several psychiatric disorders can become valid causes for marital incapacity

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of performing an accurate forensic psychiatric-psychological assessment of mental illness, which can reduce or exclude conscious ability to perform a legal act such as a marriage, leading to a declaration of nullity.

The Codex Iuris Canonici of the Catholic Church (CIC) establishes that a marriage can be annulled because of the recurrence of the consensual, and indirectly mental, incapacity to marry.<sup>2-4</sup> These cases show diagnostic and judicial difficulties that may involve forensic psychiatric experts, considering that the CIC do not provide a strictly defined psychopathological connotation.<sup>5,6</sup>

This report presents a case of a 35-year-old woman claiming for the declaration of nullity of her marriage that lasted two years. A forensic psychiatric examination was required: until the age of five, the woman was entrusted to her grandparents because her parents were unable to take care of her. She described her mother as “a Satanist” who had sex with different men and women during esoteric rites and was physically and emotionally abusive toward her. Her father was completely absent and disinterested in the family, so she was soon placed in an orphanage, then in a foster family. She reached higher education becoming a gynecologist. During University, she decided to live in a monastery because of constant fights with the adoptive parents. There, she said, she was harassed and dominated by a fellow nun, who became convinced that she acted under the influence of the devil. The nun forced her to experience several exorcisms and, jealous of her religious perfection, accused the woman of having set fire to the convent. Though the woman wanted to join the religious congregation, she met her future husband in a chat web. During the engagement, they did not have sexual contact and after the marriage, she avoided sexual encounters. She showed small emotional involvement in marital relations, stating that she got married “to fill some emotional void and to verify the validity of her possible vocational choice” for which she would have married anyone. Furthermore, she wanted to annul her marriage to found a religious order of her own.

At the psychiatric evaluation, she showed emotional instability, oscillating between reticence and exhibitionism as well as victimhood and manipulation of the setting. Hypoactivity and dysempathy were manifest in a context of pseudo-erotized, conflicting, and unstable interpersonal relationships. The expert indicated that at the time of the wedding until the present she was suffering from a pervasive psychopathological condition such as Borderline Disorder for which the woman was unable to assume the essential obligations of marriage.

This case provides attendees with a better knowledge of the several psychiatric reasons underlying the mental incapacity to perform a legal act as a marriage: personality disorders, alcohol dependences, psychosexual deviations, and psycho-affective immaturity. The social environment (family background, religious education) can influence the course of the illness and sometimes can modify the clinical picture. Thus, it is necessary not only to conduct a psychiatric examination, but also take into account the course of mental disorders, their severity, and the impact on the social functioning of the person. To establish that, it is often useful to supplement the material with psychological, neuropsychological, and imaging tests and sexual scales.

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### Borderline Personality Disorder, Forensic Psychiatric Assessment, Religious Setting

## I10 The Psychotic Development of a Personality Disorder in a Case of Double Homicide

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of criminological evaluations of mental functioning in the determination of a mentally ill defendant's responsibility and dangerousness in a criminal trial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the culprit's psychiatric condition and his/her attitude toward the life events should be assessed, especially in delusion cases.

Standard interpretations of the concept of "legal capacity" refer to being a duty bearer as well as a rights holder, including recognition before the law in a broad sense. This interpretation applies not only in the context of personal decisions, but also in the context of responsibility for criminal acts. Criminal law operates on a presumption that the capacity for crime is present, unless the defendant meets the requirements of a mental incapacity based-defense.<sup>1,2</sup>

In the United States, competency and insanity are distinguished. The first refers to the offender's present state of mind and may be raised before trial, during the trial, or after sentencing. The second refers to the defendant's state of mind during the crime and may be used as an affirmative defense if the defendant is so seriously mentally ill that he/she should not be held criminally responsible under the applicable state test for insanity.<sup>3</sup>

In Italy, the criminal responsibility of the mentally disordered offenders can be fully (Art. 88 of the Italian Penal Code) or partially excluded (Art. 89) with diminished sentences, as they can be or not be unfit to plead at the moment of the crime for impaired mental functions.

This report presents a case of a double homicide perpetrated by a married 49-year-old neighbor armed with a hunting rifle. The criminological examination found that the killer had psychotherapy when he was young for a "shyness neurosis" that stopped when he met his wife. He had several short-term employments that ended with "unlawful dismissals;" he had always perceived the work environment as hostile and mobbing. He lived in the same apartment building as the victims with whom the man argued (he had lost 20 civil lawsuits). In fact, he thought that the two victims had an agreement with the building manager who approved new works just to make them earn from commissions on sales. The day of the murder, the killer was angry after having lost yet another lawsuit. When he saw the two neighbors outside, he aimed at the "big targets" and shot them by his window. In jail, the killer wrote a letter to the victims' relatives claiming "a mobbing war" and a Mason conspiracy against him by the neighbors, the building manager, the judges, and the secret service. The forensic psychiatrist diagnosed a paranoid personality disorder with persecutory delusion, present even at the time of the murder, which partially excluded the criminal responsibility (after he was sentenced to 20 years in prison). The development of the delusion matched with his rigid attitude toward life events, his isolated and unsuited life style, the inclination toward resentment and rumination, and several attempts to "redeem" himself through the lawsuits.

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### Personality Disorders, Delusion, Homicide

## I11 Child Neglect or Chronic Diseases? An Overlap and a Matter for Experts

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**Learning Overview:** After attending this presentation, attendees will better understand that sometimes caring for a child with chronic health conditions can be perceived as a burden by the parents, leading to a higher risk of physical abuse and neglect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance for pediatricians to perform accurate investigations in order to assess if nutritional deficits in children can be ascribed to some types of neglect or to a chronic congenital disease.

Child neglect is a rising concern in industrialized countries and, despite being often underreported, it represents the most prevalent form of maltreatment.<sup>1</sup> Welch and Bonner defined two main categories of neglect: deprivation of necessities or caregiver's inability to provide for the child's basic needs (e.g., food, water, shelter, medical care, clothing, and education) and neglect or failure to provide adequate supervision and safety according to the child's developmental age.<sup>2</sup>

In the same countries, the prevalence rates of childhood chronic illnesses that fall under a specific medical discipline such as endocrinology, cardiology, gastroenterology are on the rise. In particular cancer, Diabetes type 1 (DM1), chronic infections, neurological impairment, hypoxemia from congenital heart defects, and gastrointestinal diseases (malabsorption, celiac disease, etc.) are increasing.<sup>3</sup>

In order to contribute to the knowledge of the topic, this study performed a five-year retrospective analysis of suspected neglect cases managed by the multidisciplinary "Bambi" Unit of the Pediatric Hospital "Regina Margherita" of Turin, Italy, dedicated to child abuse, between January 2015 and December 2019. Among the 998 children treated by the Unit in this period, 96 were suspected cases of child neglect. Among them, 27 children were affected by chronic health conditions: 10 young boys and 17 girls; the mean age was 4.9 years. The family status analysis identified the same number of divorced and not divorced parents. Childhood chronic illnesses included: DM1 (nine cases), congenital heart defects (three children), dermatological diseases (four cases), neurological impairment (three children), malabsorption (six cases), and recurrent infections (two children). Almost half of the cases were reported to the Judicial Authorities and social workers intervened because the parents were deemed unable to properly care for them. The most serious case involved a 12-year-old girl, affected by DM1, who died subsequent to hyperglycemic coma. Her mother exhibited severe parent-child interactional difficulties leading to inadequate insulin administration. She also displayed tentative and inconsistent parental behaviors and inefficient coping skills.

This case provides attendees with a better knowledge of some conditions, such as severe metabolic imbalance or nutritional deficits, that may result from either child neglect or chronic congenital diseases.<sup>4</sup> Sometimes both situations overlap, making it hard for health care providers to reach a proper differential diagnosis. Not only a deeper medical investigation, but also a holistic approach, is mandatory to prevent the symptoms from worsening, requiring prompt medical care and social support.

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### Child Neglect, Chronic Diseases, Parental Behavior

## I12 Autoeroticism and Depression in Lockdown From COVID-19: Accidental Death in Combined Asphyxiophilia—Autoerotic Asphyxia by Nitrogen Inhalation and Secondary Effects of Trazodone

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**Learning Overview:** After attending this presentation, attendees will know more about the accidental asphyxiating death in a depressed patient during lockdown from COVID-19.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of an unusual accidental death in a paraphiliac syndrome.

Reported here is the case of the death of young man B.D.G, ascribed to an accidental mechanical asphyxia. He was found dead by his parents in his house, supine in the bed without pants or underpants, covered only with a sheet and blanket. Around his head and neck was a handmade “device” consisting of a plastic garbage bag, a shoe string that secured it around his neck, and a tube, also stabilized with tape, connecting the bag to a large cylinder of industrial Nitrogen (N<sub>2</sub>) gas next to the bed, with the knob closed.<sup>1</sup> The plastic bag was torn by the decedent’s father, who found the body. The investigations uncovered many searches by the patient on the internet for the choice of suicide mode. The time of isolation in the house favored such research. Also found were numerous packs of drugs on the table, most of them psychiatric. After external examination of the body, a blood sample was obtained to analyze the concentration of drugs in the blood. The results showed that the only drug present in the blood of the deceased was trazodone.

Trazodone is a serotonin receptor antagonist and reuptake inhibitor used extensively as an anxiolytic in human and small animal veterinary medicine. It is also Food and Drug Administration (FDA) -approved for the treatment of depression and has been used by mental health and primary care providers for the treatment of multiple psychiatric and medical conditions.<sup>2,3</sup> Prolonged penile erection is one of the different side effects associated with trazodone use. This drug can enhance penile erection through a proposed mechanism related to the alpha-adrenoceptor blocking properties of trazodone by interference with the sympathetic control of penile detumescence.<sup>4,7</sup>

The cause of death was assumed to be suffocation caused by the plastic wrap covering the face, combined with nitrous oxide inhalation. Nitrous oxide, also known as laughing gas, has a euphoric effect and is used as a recreational inhalant drug that can be purchased legally. Suicide was suspected due to a history of chronic depression aggravated as a result of the imprisonment imposed by the COVID-19 pandemic.<sup>5,6</sup> Deaths caused by recreational nitrous oxide abuse are rare, but may occur if used in combination with a plastic bag over the head. This is the first report of accident by suffocation by external obstruction combined with nitrous oxide inhalation and use of trazodone for auto-erotic practices.

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### COVID-19, Nitrogen Inhalation, Trazodone

## I13 Family Mass Murders in Italy, 1990-2019

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**Learning Overview:** After attending this presentation, attendees will have learned about the features of family mass murders that occurred in Italy between 1990 and 2019.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding new knowledge about an uncommon type of murder that has received little attention from the scientific literature.

Family mass murder (or familicide) is a multiple-victim homicide of all, or most all, the family members. It is sometimes followed by the suicide of the killer.<sup>1-3</sup> Although uncommon, familicide is the most frequently occurring type of mass murder in Italy. The perpetrator may be motivated by a desire for revenge after a separation or perceived wrongdoing or driven by the compassionate motive to ease unbearable suffering.<sup>3,4</sup> However, the reasons behind familicide are often hard to discern even after extended criminal and psychological investigations. Research is hindered by the lack of a national database on familicide as a separate entity from any intrafamilial homicide.

In order to contribute to the knowledge of this topic, a systematic search of cases of familicide that occurred in Italy in the past 30 years was conducted. Familicide was defined as the homicide of the partner or ex-partner and one child/stepchild or more or the homicide of at least two family members of different generations.

Reports of familicides were collected through a review of the online archives of the main national newspapers from January 1990 to August 2019. When possible, data on individual events were cross-referenced for better accuracy. Inclusion criteria consisted of a minimum set of data: date and place of the event, characteristics of the killer, characteristics of the victims, and circumstances of the homicides. Data were divided into categories and compiled into a spreadsheet using Microsoft® Excel®. Categories were examined through univariate and bivariate statistical analysis.

The research identified 52 cases of family mass murder for a total of 165 victims (including 37 perpetrators who committed suicide). Of those, 96.2% ( $n=50$ ) of killers were men. In 82.7% ( $n=43$ ) of cases, the murderer was the male head of the family (father/husband/stepfather). In 30.8% ( $n=16$ ) of perpetrators fell into the 41–50 year old age group while 21.5% ( $n=11$ ) equally belonged to the 31–40 and the 51–60-year-old age groups. The killer was married to one of the victims in 78.8% ( $n=41$ ) of cases and had a job in 61.5% ( $n=32$ ) of cases. The total number of victims was three in 55.8% ( $n=29$ ) of cases. Adult victims (>18 years old) were evenly distributed across the age groups. Underage victims (<18 years old) were <12 years old in 59% ( $n=32$ ) of cases, with a mode of 4 years old. Wives/ex-wives were 43 out of 74 adult victims (58.1%). The family group was of non-Italian ethnicity in 11.5% ( $n=6$ ) of cases. Familicides were slightly more frequent in wintertime ( $n=19$ ; 36.5%) and mostly occurred in southern Italy ( $n=24$ ; 46.1%). Another 88.5% ( $n=46$ ) of familicides took place in the family home or the home of a family member. Perpetrators mainly used firearms ( $n=24$ ; 46.1%), knives or cutting instruments ( $n=12$ ; 23.1%), blunt objects ( $n=6$ ; 11.5%), or a combination of instruments ( $n=6$ ; 11.5%). Overkill was reported in 44.2% of cases ( $n=23$ ). The murderer committed suicide in 71.1% ( $n=37$ ) of cases and attempted, but failed, in 11.5% ( $n=6$ ) of cases. Favored methods of suicide were firearms ( $n=19$ ; 51.3%), knives or cutting instruments ( $n=6$ ; 16.2%), and hanging ( $n=6$ ; 16.2%). Prevalent family contexts were abnormal life experiences ( $n=13$ ; 25%), separation ( $n=12$ ; 23.1%), and prolonged family conflict ( $n=9$ ; 17.3%).<sup>5</sup> In five cases (9.6%), no warning signs were reported. A statistically significant association ( $p$  value=0.04) was found between overkill and instruments other than firearms.

In conclusion, this study identified warning signs and at-risk family contexts that can potentially result in a familicide. Family-centered policies are needed to improve support for people with mental health problems or financial difficulties. Public health authorities, social services, and law enforcement should cooperate in a strategy for early intervention based on risk factors. Further research on familicide in Italy and other countries should be carried out, especially to better comprehend the alarming cases where no warning signs could be detected.

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### Familicide, Family Mass Murder, Warning Signs

## I14 The Perpetrator-Victim Bond in Matricide: An Unusual Case

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**Learning Overview:** After attending this presentation, attendees will better understand the specific purpose of the criminological investigation regarding the unusual nature of the perpetrator-victim bond in matricide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that mental illness is not the only variable related to matricide, and it could not be enough to explain the crime.

In Italy in 2018, one homicide out of two (49.5%) had been committed by relatives. Among the family members involved in homicides (the couple, the sons, the parents, the siblings, and the other relatives), the couple is the most dangerous relationship accounting for almost half of the murders in Italy from 2000 to 2018.<sup>1,2</sup>

Matricide has always been considered one of the most abhorrent crimes, but is a rare event. In Italy, among matricides and patricides, 57.1% of victims are mothers killed by their sons. Matricide seems to be more common among individuals with psychiatric disorders (49.2% of cases), especially schizophrenia or other psychoses. The motives involve disputes and disagreements (23.3%) arising from co-existence often forced and made difficult by marginalized now-adult children unemployed or addicted to drugs, alcohol, or gambling. Economic motives account for 12.7% of matricides. The victim's discomfort or the anguish and fatigue of having to look after a sick parent (physically or mentally) was found as the prevalent motive in 8.2% of cases. According to the literature on matricides perpetrated by a son, most of the perpetrators are single adults with an intense relationship with their mother, a lack of interest in other women, a feeling of social inferiority, and an absent or passive father.<sup>3,4</sup>

This report presents a case of an attempted matricide perpetrated by a homophilic dyad composed of the victim's second daughter and her mistress. The roles of the instigator and the executor were progressively confused until they inverted. The victim was a 55-year-old woman assaulted in her home by her daughter's mistress, a 28-year-old woman, who tried to kill her using first a hammer and then a knife. Only chance and the victim's prompt reaction allowed her to survive. The instigator was the daughter, a 24-year-old university student, who lived with her mother. The two accomplices were arrested the next day. The motive of the criminal project was two-fold: to prevent the mother from discovering her daughter's false degree and to avoid the risk of the victim becoming aware of the homophilic relationship with the executor, always opposed by the mother. It was also found that the daughter had tried to kill her mother many other times, but the attempts were never realistic (e.g., hiring an assassin to run over or shoot the victim, paying a sorcerer to poison her by a "satanic fluid," etc.).

This case of matricide presents with atypical characteristics and interesting particularities found at criminological investigation of the aim and the type of the crime, planned "in concurrence," but carried out "by proxy."<sup>5</sup> The particular dynamics of the mother-daughter relationship and the unique personalities and life experiences of the instigator/executor couple are the real key to this case of matricide.

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### Matricide, Personality Disorders, Homicide

## I15 Could a Psychogenic Death Occur? The Scientific Analysis of a Mysterious Biochemical Process

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**Learning Overview:** After attending this presentation, attendees will understand the significance of psychogenic death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by analyzing the hypothesis of a dysfunction of the dopaminergic system as a cause of psychogenic death.

Several historical sources have been reported about mysterious cases of death not related to organic pathology. These cases have been called “give-up-itis,” described as cases of people who let themselves die. Historically, cases of prisoner soldiers in concentration camps during the various war conflicts that characterized the past century have been found. Other similar cases have been reported about survivors of shipwrecks or plane crashes.

Psychogenic death was not found only in such catastrophic events. In literature, there are many examples about hospitalized patients whose death was attributed to psychogenic death. In particular, in 1979, Stumpfe reported the case of a man who, the day after a surgical operation that he perceived to be unsuccessful, showed symptoms of regression, discharge, passivity, and apathy and died in a few hours.<sup>1</sup> Subsequently, an autopsy was performed with histopathological and toxicological analysis that showed no clear cause of death.<sup>1</sup> The most widely accepted etiopathogenetic theory concerns a dysfunction in the central nervous system. The nerve centers involved in this event appear to be the inter-neuronal circuits at the level of the frontal, prefrontal, and cingulate cortices and their interconnections with the basal ganglia. The neurotransmitter involved is dopamine. In particular, it seems that the etiology depends on a depletion of dopamine in the anterior cingulate circuit.

The dopaminergic system is essential in determining the response to an external stimulus and the associated emotions and behavior. It has been scientifically demonstrated for decades that people with low levels of dopamine tend to be apathetic, up to a real motor slowdown. The large part of psychiatric disorders are treated through a modulation of dopaminergic transmission and drugs such as clozapine are fundamental in reducing suicidal risk.<sup>2</sup> Some stages in psychogenic death have been identified. It all begins with a trauma that induces psychological withdrawal and apathy. Then there is a state of abulia up to psychic akinesia. The last phase is represented by death. From the literature review, it emerged that psychogenic death is not an acute event, but generally requires several days to occur and chronologically follows the steps described above. This event must not be associated with a common psychiatric illness such as depression. Some studies support the reversibility of this event. Scientific studies have shown that a psychotherapeutic and psychopharmacological approach can allow a recovery. The key element in understanding this disorder is subjectivity. In fact, not all the imprisoned soldiers or all the survivors from catastrophes manifest this syndrome. In these cases, a psychological substrate may coexist which favors its manifestation.<sup>3</sup>

Therefore, psychogenic death cannot be denied from a scientific point of view. Since this is a syndrome unrelated to organic alterations, an issue must be raised: the role of autopsy. In fact, it is based on a biomolecular process with no macroscopic manifestations, thus causing great problems for its diagnosis. From the forensic point of view, a thorough investigation about other causes and the exclusion of other probable and demonstrable causes of death is necessary, but above all, it is fundamentally necessary to analyze the clinical history of deceased subjects. In these cases, it is mandatory for the forensic pathologist to evaluate the personal history of the subject with possible presence of stressors such as to induce the degenerative biochemical cascade described to make diagnosis. At the same time, the entire international medical community must be updated about this issue since it is a reversible phenomenon through the increase, in the aforementioned neuronal circuits, of the levels of dopamine. It would be useful to create possible criteria that can also be used in the clinical and forensic field to prevent the event when it is in a reversible phase or to detect a postmortem diagnosis.

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### Forensic Science, Psychiatry, Death

## I16 Face Similarity Linkage: A Novel Biometric Approach to Sexually Motivated Serial Killer Victims

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**Learning Overview:** The goal of this presentation is to showcase another investigative tool for crime linkage by using facial biometrics of serial killer victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing new techniques to investigators in serial killer cases when all other investigative tools are exhausted. This technique, using facial biometrics on victims, can be used as a building block to help toward a conviction.

Crime linkage can be difficult if there is no physical or genetic evidence left at a crime scene; therefore, other techniques need to be used to link crimes to an offender.<sup>1</sup> The type of offender committing these crimes can be ascertained by assessing the crime scene, the victims, and distinguishing features that link crimes and therefore the killer. This study focuses on the crimes of sexually motivated serial killers. Owing to the sexual motivation of attacks, some serial killers may have a particular “type” or appearance of prospective victims that they are drawn to. Therefore, similarities in facial biometrics of victims may be useful to help link multiple victims to a single offender. Being able to make the connection to a killer by linking the victim’s facial measurements stands as a useful investigative tool. Biometric approaches may be used as a means for crime linkage in the absence of physical evidence at a scene. The current study provides a background on crime linkage, typologies of serial killers, and victimology in order to set the scene for the application of biometric methods as a novel linkage measure.

The current research was undertaken to propose a technique, termed Face Similarity Linkage (FSL), to evaluate whether victims of a serial killer have statistically more similar facial measurements than a randomly chosen person of the same gender. To test this, three of Ted Bundy’s victims were randomly selected and anatomical landmarks were located and measured to produce proportionality indices of their faces. A random subject from an online database was used as a comparison. The results showed there were no statistically significant differences between the three of Bundy’s victims; however, there was significant difference between 11 of the 17 facial measurements of Bundy’s victims when compared to a random person. This research serves as a proof of concept that, with more advanced means of data collection, FSL may be a useful tool for law enforcement for linking serial homicides. The current method is relatively novel and in need of expert systems interfaces to improve speed and application, which is outlined in the current study.

The prospect that any investigator can use this method due its simplicity, low cost, and speed is a great benefit. There is no need for extensive training or equipment; this research may be replicated with printed images, a ruler, and pen. Clearly the proposed method is a tool, not a complete solution. Other factors including time and geography of potential victims should always be considered. However, as a relatively quick method of matching, this method has potential for application to many unsolved and future crimes. This potential may be further progressed by the adaption of the current manual procedure to an automated, *in silico* solution with the assistance of software engineers and experts.

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### Serial Killer, Biometric, Face Similarity Linkage



## I17 Psychosis and Psychopathy: Two Conditions That Have Determined the Form and Function of the Insanity Defense

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**Learning Overview:** After attending this presentation, attendees will have a greater appreciation of how not one but two conditions, psychosis and psychopathy, have determined the form, function, and political acceptance of the insanity defense.

**Impact on the Forensic Science Community:** With a parallel understanding of how both psychosis and psychopathy have historically and continue today to pertain to rational and moral capacities respectively, this presentation will impact the forensic science community by helping attendees gain a more refined conceptualization of the presumptions that define and justify the insanity defense.

For over one-and-three-quarter centuries, enduring conceptions of the function and purpose of the insanity defense have basically involved two conditions, psychosis and psychopathy. Yet these two conditions are seldom analyzed together. This presentation will do so and will argue that rational capacity, more than cognitive and moral capacities, is fundamental to the importance and continuing existence of the insanity defense in the United States.

A contrary conception of criminal responsibility would allow the defects of psychopathy, without the psychotic loss of rationality, to qualify for the insanity defense. Psychopathy and related conditions such as antisocial personality disorder have long been a part of the scientific discourse on criminal responsibility. In 1838, in *The Medical Jurisprudence of Insanity*, Ray argued that offenders with moral insanity should not be held responsible for their crimes.<sup>1</sup> Rather, to protect the public, they should be civilly committed to a mental hospital. One hundred years later, Gregory Zilboorg made essentially the same argument.<sup>2</sup> Now in the 21<sup>st</sup> century, Stephen Morris maintains that defendants with severe psychopathy should be found not criminally responsible for their criminal acts because they cannot make moral decisions.<sup>3</sup> He too recommends civil commitment rather than criminal punishment. Studies indicate that individuals with psychopathy can intellectually solve moral dilemmas, but they are not motivated to behave morally because of a Central Nervous System (CNS) defect that may involve the amygdala.<sup>4,5</sup>

This study argues that a push toward broadening the insanity defense to include psychopathy could strengthen opposing arguments and sentiments against the insanity defense.<sup>6</sup> Growing disdain for the insanity defense can lead to it becoming less available for those who are most deserving of this excuse—defendants who acted out of psychotic irrationality. In other matters, the law follows a principle of normative functionalism.<sup>7</sup> Individuals are not expected to do what they cannot do. The question of the quality and severity of a defect needed to provide an excuse is normative, determined by rules established by government reflecting both scientific knowledge and public sentiment. The Supreme Court has required rationality as essential to trial and execution competence.<sup>8,9</sup> From this perspective, even as psychopathy influences one's thinking and behavior, it is reasonable for the consequential threshold to be rational capacity beyond simple factual awareness.

This past year, in *Kahler v. Kansas*, the United States Supreme Court found that a state's abolition of its insanity defense was constitutional because the state afforded defendants a *mens rea* defense, which requires cognitive capacity.<sup>10</sup> Moral capacity, as required by most state insanity standards, is not constitutionally required. Unexpected, unnecessary, and scientifically erroneous was the Court's reference to psychopathy in a footnote to further support its holding. The Court reasoned that the moral capacity insanity standard was not fundamental because several states did not include moral capacity in their insanity standards. State standards were too diverse for any one standard to be considered as fundamental. Moreover, several states included the American Law Institute's Second Paragraph excluding psychopathy from consideration for the insanity defense, which the Court opined was inconsistent with the defendant's position that a moral capacity insanity test was fundamental.<sup>11</sup> The Court's premise that those who repeat their criminal conduct, even those who are psychopathic, are unable to make moral decisions was erroneous. The Court's more serious error, however, was its failure to consider rational capacity, which is essential to all insanity standards and historically fundamental as well, a capacity that can be profoundly defective in psychosis, but not in psychopathy.

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### Forensic Science, Insanity Defense, Rational Capacity

## I18 The Insanity Defense in the Contemporary Context

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**Learning Overview:** The goals of this presentation are to: (1) analyze how psychopathic disorders were favored and disfavored in the United States as conditions qualifying for the insanity defense and commitment; (2) describe Kansas' attempt to abolish the insanity defense and analyze the constitutional challenges posed by *Kahler*; (3) identify a group of individuals adjudicated Guilty Except Insane (GEI) in Arizona who are at risk of transfer from a state hospital to a correctional setting; and (4) understand the impact of *mens rea* insanity defenses on dispositions of mentally ill defendants.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that a thorough understanding of recent changes in the jurisprudence of the insanity defense better prepares practitioners addressing mental illness defenses and policy makers striving for rational, fair, and effective procedures impacting mentally ill offenders.

The insanity defense is under attack. Since the trial of John Hinckley, Jr., state legislatures have enacted statutory changes attempting to place limits on the insanity defense by introducing processes designed to narrow who is eligible.<sup>1</sup> Despite this trend, insanity defenses are proposed for defendants with psychopathic disorders.

This presentation will discuss the *psychopathy paradox*, which recognizes that although psychopathic disorders per se do not qualify for extended insanity commitment, psychopathy contributes to risk assessments which disfavor hospital discharge from insanity commitment.<sup>2,3</sup>

This presentation next explores statutory changes made between 1979 and 1996 that resulted in abandoning traditional insanity defenses in Montana, Idaho, Utah, and Kansas. Instead, these four states, adopted *mens rea* statutes that require an individual be unable to form the specific intent necessary to commit a crime in order to be found not guilty as a result of mental illness.

The presentation will review limited empirical data from each of the four *mens rea* states with a focus on the impact of these changes on individuals with severe mental illness who are involved in the criminal justice system.<sup>4-6</sup> In light of this impact, this presentation will review the recent United States Supreme Court case of *Kahler v. Kansas*, which determined that the Kansas *mens rea* insanity defense was sufficient and that decision, by extension, would apply as well to the other three *mens rea* states.<sup>7</sup>

Yet another alternative to traditional insanity defenses, this presentation will explore Arizona's GEI verdict that provides a unique mechanism to address offenders with psychopathy. Arizona allows its Psychiatric Security Review Board to determine that an individual who is found GEI for certain crimes is dangerous, but no longer in need of psychiatric treatment.<sup>8,9</sup>

The presentation will conclude with a plea for organized psychiatry and psychology to develop research programs that focus on the effects of statutory changes in these states and others that may adopt similar provisions. This research can inform debates about statutes and focus on what happens to severely mentally ill persons in these areas of the criminal justice system.

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### Forensic Science, Insanity Defense, Psychopathy

## I19 An Overview of the Cultural Defense

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**Learning Overview:** The objective of this presentation is to review the “cultural defense” and discuss the role that forensic evaluators may play in assisting the courts in understanding such a defense. This presentation will discuss the intersection of mental health law, the criminal justice system, and psychological theory as they relate to cultural defenses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that mental health professionals can be pivotal in the evaluation of defendants bringing forth the cultural defense to explain their violent crimes.

The cultural defense has historically been raised by immigrants and used to negate *mens rea* and/or reduce culpability in cases involving violent crime. For example, the cultural defense has been used to reduce culpability in the context of honor killings. The cultural defense could potentially be used to understand the actions of defendants whose behaviors were influenced by the cultural norms from the community with which they identify even though viewed as criminal in the United States.

There are three paramount cases in which the cultural defense has been raised: *People v. Poddar*, *People v. Kimura*, and *People v. Chen*.<sup>1-3</sup> This presentation will focus on how the cultural defense has historically been used and implications for future use.

In *People v. Poddar*, the defendant, Prosenjit Poddar, was rejected by a classmate who he believed he had a relationship with.<sup>1</sup> Upon her rejecting him, he murdered her and subsequently argued that his cultural background impacted his perception of their relationship. He raised a cultural defense through the diminished capacity defense. He argued that at the time he committed the homicide, he was experiencing cultural disorientation and extreme emotional distress as a diagnosed paranoid schizophrenic, which rendered him incapable of fully understanding the nature of the crime that he committed.<sup>4,5</sup>

In the *People v. Kimura*, the defendant, Fumiko Kimura, drowned her children and attempted suicide upon learning of the infidelity of her husband.<sup>2</sup> Kimura raised the Japanese concept of *Oya-ko shinju* (parent-child suicide) whereby it was a more intense crime for a parent to commit suicide and abandon her children than to murder the children.<sup>5,6</sup>

In the *People v. Chen*, the defendant, Dong Lu Chen, bludgeoned his wife to death due to her alleged infidelity<sup>3</sup>. Chen was a Chinese immigrant and argued that cultural factors made him unable to formulate *mens rea* for the premeditated murder because in Chinese society he would have been stopped by the community from murdering his wife due to her infidelity. Chinese society would have recognized the blemish on his reputation and his now tarnished legacy due to the actions of his wife, but the society at large would have prevented him from committing murder. The judge in this case readily acknowledged that Chen, while a murderer, was also a victim of American society which failed to properly assimilate him and therefore failed to stop him from murdering his wife.

The aforementioned three cases describe how the cultural defense has been invoked in the forensic community by immigrants seeking to attribute violent behaviors and criminal acts to the cultural norms and traditions used in their respective ethnic groups. Although generally unsuccessful in avoiding punishment in United States courts, the cultural defenses have implications for how we understand the way criminal culpability is viewed through the mono-cultural lens of the American criminal justice system.

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### Culture, Defense, Psychology

## I20 The Management and Epidemiological Data of SARS-COV-2 Emergencies in Prisons: The Italian Model

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**Learning Overview:** After attending this presentation, attendees will understand how the COVID-19 emergency was managed in Italian prisons and the effectiveness of the prevention measures until now.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the effectiveness and criticalities of the strategies adopted in prisons that can represent places of infectious outbreaks that are difficult to contain. Also emphasized is the need for mental health protection of prisoners who are already at higher risks of depression and suicide compared to the general population.

The SARS-Cov-2 pandemic is currently a major worldwide public health problem. In prison, the management of preventive measures is difficult due to overcrowding and inmates' and officers' close physical contact. There is also less access to care than in community settings. For this reason, the adoption of strategies aimed at containing infections in these places is mandatory. In addition to the infectious disease problem, it is necessary to consider that prisoners are more subject to depressive episodes and an increased risk of suicide. This increase is due to the conditions of deprivation of personal freedom and the difficult living conditions inside prisons. World Health Organization (WHO)/Europe has published guidelines that contain useful information for prison staff and health care providers who work there.<sup>1</sup> They advise on how to prevent and manage a potential epidemic outbreak and highlight the importance of human rights that must be respected. Above all, access to information and support for mental illness are essential. Each country has a responsibility to increase its level of preparation, to be alert, and to respond to identify, manage, and treat new cases of COVID-19. Recognizing that there is no single approach for handling COVID-19 cases and outbreaks, they should adapt their approach to the local context.

In Italy, the Decree of March 17, 2020, introduced provisions that concern some 4,000 prisoner,; providing for the possibility for home detention for those who have less than 18 months' sentence to serve.<sup>2</sup> The measures were applied by the supervising magistrate not only at the request of the detainee, but also by the public prosecutor or the prison governor.

As a result of this provision, the number of inmates in Italian prisons (which remain in overcrowded conditions) dropped by 13.9%. Furthermore, during the first phase of the epidemic, the crime rate in Italy was reduced with a consequent lower number of detainees. This fact is certainly due to the restrictions on movements during the lockdown.

In Italy, the first case of contagion occurred in mid-March, a significant delay compared to the rest of the world. So far 119 inmates have been infected and 162 cases of infection have been registered among the staff. COVID-19 deaths have been eight in total: four inmates, two doctors, and two prison police officers. Seven deaths occurred following the riots in prison that broke out at the beginning of the emergency phase. In most prisons, there was not even a single case of contagion. The relative isolation of prisons from society was a sure factor of protection and the measures adopted certainly played an important role in containing the epidemic. With regard to psychological support, the inmates were granted a psychology service via electronic means to alleviate anxiety and concern about the emergency. Finally, the inmates were allowed to use smart phones and Skype® to maintain contact with family members.

Regarding the number of suicides, in the first five months of 2020, there was an increase in cases compared to 2019.<sup>3</sup> It is possible that this data may be related to the effects of the pandemic, which probably increased feelings of isolation and hopelessness. Overall, the management of the emergency in Italian prisons has certainly allowed a low rate of infection and death compared to other countries. It is certainly necessary that psychological support is implemented to allow the containment of anxiety and depression disorders and for the prevention of suicide.

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### Forensic Sciences, COVID-19, Prison

## I21 Confinement and Psychotic Decompensation: A Forensic Perspective

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**Learning Overview:** The goals of this presentation are to introduce a broader understanding of mental pathology and, specifically, of psychotic disorders and provide the ability to empathize with the patient/client.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing active listening of the patient and increasing the efficiency and honesty in professional intervention (ethics and correctness in the professional practice).

There has been a recent increase in consults for clients who are suffering from acute symptoms of psychosis, including schizophrenia, delusional disorders, bipolar disorder, and others. While consultants often perform evaluations of this type, there has been an unusual increase in the amount of consults that have occurred during the COVID-19 pandemic.

As is well known, symptoms of psychosis are difficult to treat and patient adherence to pharmacological interventions is poor. The support of first-degree relatives and involvement in their care significantly increases adherence and improves outcomes. With the help of these family members, the patients can be linked to care and services they may not otherwise receive. In direct contrast to this, patients without primary family support are the most vulnerable in the system, become fragile, and are more prone to psychotic decompensation and reactive psychosis. Ultimately, it becomes very difficult for providers to intervene in a meaningful way with these populations.

During the COVID 19 pandemic, populations have been ordered into confinement and social isolation for periods of at least two months and many times longer. This has exposed already vulnerable populations to increased stressors and led to decreased compliance, whether volitional or due to lack of resources. Those individuals who have been isolated from their family supports who may otherwise maintain stability have seen an increase in psychotic decompensation along with the patients without primary support.

There is evidence that these decompensations have led patients to seek legal counsel as a result of harmful pathological thought content, including delusions and hallucinations. Because the thought content of those suffering from psychosis (especially those in an acute crisis/psychotic decompensation) is disorganized, they are difficult to advise. Whether their psychosis presents as part of a primary psychotic disorder or a personality disorder, these delusions are typically rigid and indelible.

This creates significant challenges for legal counsel, especially when attempting to explain that they have no legal claim. Attorneys must bear in mind their work economy, economy of emotional involvement in legal matters, the financial concerns of the client, and the client's reputation. No less significant is their reputation as a firm for bringing cases that have no legal standing.

It is important for attorneys to learn language to watch out for, including statements such as "someone is chasing me," "they have hurt me," "they have put microphones under the tables in my house or in the breadcrumbs." When asking clarifying questions such as "Who is persecuting you?," "Who hurts you?," "Who is bugging you?," the answers are likely to be vague, imprecise, and elusive. Rarely will a clear and logical story be elucidated. These things may signal the importance of consulting a psychiatric expert.

Overall, the pandemic has put significant stress on the world population, and individuals who would otherwise be able to compensate have exhausted their resources. The legal field and forensic and criminal psychology have felt that increased stressor through increased consults. This is likely to continue for some time into the future.

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### Confinement, Psychotic Decompensation, Forensic Psychology

## I22 “Folie à Deux” (Simultaneous) and Deliriant Induction to Murder

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**Learning Overview:** The goal of this presentation is that attendees are updated with the concept of “Folie à deux” and its clinical-symptomatological phenomenology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by imparting a greater degree of knowledge of the psychopathological phenomenon of “Folie à deux” and of the various existing subtypes. It is intended that this presentation increases and/or enhances the diagnostic capacity of possible clinical cases of “Folie à deux” and their forensic repercussions.

**Introduction:** At the end of the 19<sup>th</sup> century, Lasagne and Falret described the psychopathological phenomenon known as “Folie à deux”. Before them, authors like Baillanger and De Saule already developed the first descriptions of this same mental phenomenon.

According to Lasagne and Falret, in the “Folie à deux”, the patient with a psychotic disorder manifests his delusional disorder by adopting an active role in imposing it on a (passive) subject belonging to his most immediate social circle. For the phenomenon of “Folie à deux” to materialize, it is necessary that both the active subject (with delusional disorder) and the passive subject (without delusional disorder) remain isolated for a long time from any external influence; a circumstance that encourages both members to share emotions and feelings (such as fears or needs). Likewise, the delusional content must be contextualized within the limits of what is possible, with sufficient coherence to be acceptable. Although this phenomenon does not exclude the male sex, it tends to be more frequent in the female population. From the psychotherapeutic point of view and as an interventionist methodology, the separation of the two subjects is the usual procedure, in most cases resulting in the remission of the delusional symptoms in the passive subject.<sup>1</sup>

In subsequent years, four subtypes of the general syndrome were established: (1) Folie imposée: the primary subject transfers the delusions to the secondary, passive, and less intelligent element. The delusional ideas of the receiver disappear after the separation; (2) Folie simultanéé: delusions occur simultaneously, but independently, in both people who live together. Both are predisposed to suffer from a psychotic illness. As there is no primary subject, separation by itself would not improve the picture for either of them; (3) Folie comuniqueé: the recipient develops psychotic symptoms after a variable period of time, and these symptoms end up having their own evolution. The separation does not influence the content of the painting; (4) Folie induit (variant of the previous one): a subject who is already delirious enriches her delusions with those of another patient while both are in intimate contact.<sup>1</sup>

In the present case, an 81-year-old man, suffering from chronic Delusional Psychotic Disorder, in collusion with his son (also suffering from Paranoid Schizophrenic Disorder) perpetrated the murder of the daughter of a neighbor on the landing of his home with edged weapons (a picket and a serrated knife). The victim was attacked on the landing of the perpetrator’s home and required stitches from 100 stab wounds throughout her body. The aggressor had previously submitted multiple neighborhood complaints for conflicts related to coexistence in the community.

**Method:** (1) Directed clinical-expert interview (anamnesis) with the informed person with a total investment of approximately four hours in a penitentiary center in the province of Barcelona, Spain; (2) interview with the son of the informed; (3) updated administration of psychometric tests in order to assess their mental and psychopathological state (the Minnesota Multiphasic Personality Inventory [Mini-Mult] and the Millon Clinical Multiaxial Inventory, 3rd edition [MCMI-3]); and (4) analysis of the clinical and legal documentation provided.

**Results:** The results obtained from the tests administered suggest that the aggressor is compatible with a long-standing chronic Delusional Psychotic Disorder. Likewise, and because of the interview with the informed son, the existence of symptomatological clinical phenomenology compatible with a paranoid-delusional schizophrenic disorder is observed.

**Discussion/Conclusions:** Although father and son suffered from psychotic disorders, the perpetrator was in the middle of an acute psychotic break at the precise moment of committing the crime. The phenomenon of “Folie à deux,” in the present case, fulfilled the criteria established by Regis and Montyel as “Folie Simultanée,” while the delusions occur simultaneously, but independently in both people who coexist; both predisposed to suffer from a psychotic illness. As there is no primary subject, the separation by itself would not improve the picture of either of the two.<sup>1</sup> According to the judgment, the informed and subject under study were considered “criminally responsible for a CRIME OF MURDER defined above, with the concurrence in his performance of the INCOMPLETE EXEMPTION OF PSYCHICAL ALTERATION and the mitigating repair of the damage to the penalty of fifteen years in prison with absolute disqualification during the time of the sentence as well as the security measure of internment in a center appropriate to his mental situation for a period of FIFTEEN YEARS, a measure that will be fulfilled before the sentence.”

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### “Folie à Deux”, Deliriant Induction, Severe Mental Disorder

## I23 A Case Study of Amok Syndrome and Intermittent Pathological Impulsiveness

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**Learning Overview:** The goal of this presentation is to keep attendees updated on the concept of Amok Syndrome with a practical case. After attending this presentation, attendees will know how to distinguish between Amok Syndrome and the rest of the modalities of multiple deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of understanding the *modus operandi* of a subject (practical case) who perpetrates the murder of four victims from the harmful influence of an Amok Syndrome.

**Introduction:** Amok Syndrome is characterized by an acute episode of violent and uncontrolled behavior that the person does not remember later.<sup>1</sup> Unlike Intermittent Explosive Disorder, Amok presents as a single episode rather than a pattern of aggressive behavior and is often associated with prominent dissociative traits. Likewise, although it is traditionally observed in Southeast Asian countries, Amok cases have also been reported in Canada and the United States.

The episode is triggered by the perception of a lack of respect or an insult and seems to be prevalent among men. The episode is frequently accompanied by paranoid ideas, automatism, amnesia, exhaustion, and a return to the premorbid state after the episode. On some occasions, Amok can appear during the presentation of a brief psychotic episode or constitute the beginning or an exacerbation of a chronic psychotic process. Also, the form of presentation is abrupt and without a prodromal period.

In this sense, in Amok Syndrome, aggressive behavior can appear in the absence of a mental disorder. Behavior is distinguished from intermittent explosive disorder by the existence of incentives and gains from the aggressive act. In the forensic context, people can simulate an intermittent explosive disorder to avoid responsibility for their behavior.

The present case involves a 58-year-old male, a native of a municipality in the province of Girona, Spain, with a single marital status. He is the oldest of three brothers, has a basic academic level, and has been a bricklayer for about 30 years. The accused was visited as a preventive inmate in a nearby penitentiary in the aforementioned province while waiting to be tried for a consummate crime of four murders.

The *Iter Criminis* of the reported was as follows: two murders in a bar in a municipality in the province of Girona. The victims were father and son and were shot and killed by shotguns and two murders perpetrated with a shotgun, 15 minutes after the first murders, in a savings bank in a nearby municipality. The victims were workers at the bank branch.

**Methods:** (1) Directed clinical-expert interview (anamnesis); (2) updated administration of psychological tests in order to evaluate their psychopathological state; and (3) analysis of the clinical and legal documentation provided.

**Results:** The results obtained suggest that the aggressor was compatible with an Intermittent Explosive Disorder with a subclinical modality of pathological impulsivity of Amok Syndrome at the precise moments and instance of the perpetration of the events that occurred and that they were the subject of legal-legal debate.

**Discussion/Conclusions:** The aggressor presented compatibility of psychic dysfunction of pathological impulsivity, originally badly channeled by the anger he presented when he did not tolerate the frustration of not being properly paid professionally. Likewise, and after about 15 minutes of perpetrating the first criminally impulsive act, the perpetrator did not tolerate his frustration when claiming an amount in debt from the savings bank and he perpetrated his second criminal act, killing two more people on the spot with a firearm (shotgun). The criminal-criminological behavior was the product of a set of psychopathological factors: (1) pathological impulsivity (understood as Impulsive Control Disorder, submodality of Intermittent Explosive Disorder); (2) Mixed Personality Disorder; and (3) neurocognitive impairment due to age and a low academic level.

### Reference(s):

1. American Psychiatric Association (1995). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*. Washington. Versión española de la cuarta edición de la obra original en lengua inglesa. 1995. MASSON, S.A. Barcelona (España).

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### Pathological Impulsivity, Intermittent Explosive Disorder, Amok Syndrome

## I24 A Case Study of Murder and a Criminal Personality in a Trained Elite Military Man

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**Learning Overview:** After attending this presentation, attendees will know how to contextualize the aggressive behavior of a subject who performs elite military activities. The goal of this presentation is for forensic experts in mental health to bear in mind that elite military personnel can present “short-circuit behavior” based on the situations they see as critical to their own integrity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the increase in diagnostic competence of this type of criminal conduct, as well as the ability to appreciate what type of profile can be potentially harmful (or dangerous) for civil society.

**Introduction:** This presentation aims to explain the basic personality traits of an expert subject of an elite military discipline who perpetrated an assassination. The accused man was a sniper specializing in special missions abroad.

A 24-year-old single male, a native of Eastern Countries, with an academic level equivalent to secondary studies who was adopted at the age of 8 years old had a history of adverse psychosocial problems during his childhood, including family domestic violence up to the age of 8 perpetrated by his biological father; violence in the boarding school where he was admitted as a result of domestic violence and violence against women by his father; low school motivation; aggressive behavior problems derived from being linked to a Neo-Nazi group; a history of Attention Deficit Hyperactivity Disorder (ADHD), without unrelated clinical documentation; and a deceased (biological) father due to chronic alcohol consumption.

When the events occurred (murder at the victim’s home), the conflict originated as a result of an aggressive discussion with a neighbor. The aggressor stabbed him 30 times. The aggressor worked as an elite professional soldier (soldier/sniper in La Legión) (from a Western European country), belonging to a section specializing in anti-terrorism. As a result of this act, the aggressor was admitted to a penitentiary in Madrid, Spain, for a crime against independent human life.

**Method:** For the development of the study of the personality of the accused person, the following methodology was followed: directed clinical-expert interview (anamnesis); administration of psychological tests; and analysis of the clinical and legal documentation provided.

**Results:** The results obtained are compatible with the fact that the aggressor presents: absence of serious and / or severe mental disorder; presence of a personality disorder; presence of serious dysfunctional personality traits of emotional instability (borderline-limit) on a paranoid, anankastic-obsessive and dependent basis; and Simulation Index (SIMS) not significant.

**Discussion/Conclusions:** At the time of perpetrating the events, the accused belonged to an elite military unit: sniper expert in anti-terrorist objectives belonging to the Army Corps of the Legion and trained in hand-to-hand combat training.

At the time of the murder, the aggressor presented an acute and critical psycho-emotional alteration moments prior to the commission of the event. This was a result of the precursor events that he experienced as harmful to his personal and physical integrity. The victim “came back with a knife and he put his right hand up to defend himself while the victim wanted to stick it in his head [sic]”, according to the statement of the detainee before the judge.

The aggressor’s behavior is consistent with him experiencing a harmful impulsivity, typical of an aggressive or hostile paranoid exacerbation (Cluster A and B personality traits). He suffered an alteration of his consciousness and his capacity for self-criticism of sufficient intensity to break the inhibitory mechanisms of their conduct. This manifested itself in the harmful conduct in which it was derived and his inability to assess, in those precise moments, the scope of the fatal consequences that the perpetration of said harmful action entailed.

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### Pathological Impulsiveness, Personality Disorder, Murder and the Military



## I25 Uxoricide and Mental Disorder: A Retrospective and Descriptive Study of 30 Patients Hospitalized in a French Secure Unit Over a 22-Year Period

*Aline Quenault\*, Groupe Hospitalier Paul Guiraud, UMD Henri Colin, Villejuif, Val de Marne 94806, FRANCE*

**Learning Overview:** The goal of this presentation is to provide an analysis of the psycho-criminological profile of men suffering from psychiatric disease who attempted to kill or murdered their wives, also known as uxoricide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the understanding of uxoricide in cases of mental disease.

**Background and Goal:** Uxoricide, or spousal homicide, is defined by the murder of a wife or ex-wife and in France has long been considered a crime of passion committed by husbands when wives were unfaithful. Currently, conjugal homicides are frequently discussed in scientific reviews as domestic violence. There is a lack of clinical data about offenders suffering from a serious mental disorder, as defined by Hodgins, as they are still regularly described in a dichotomy between paranoid disorder and melancholia disorder.<sup>1</sup> The purpose of this present work is to identify the main characteristics of men with mental disease who kill their wives.

**Method:** The sociodemographic, clinical, and forensic characteristics of all men diagnosed with mental disease who committed an uxoricide and were admitted to the French Henri Colin secure unit between 1996 and 2018 (30 patients) were studied as was data about the victims. This is a retrospective and descriptive study.

**Results:** This study found a higher average age than other intra-family murderers (mean age of 36 years old), a lower level of education, and a lack of professional integration. Most subjects had a psychiatric history (76.67%); the main diagnosis was schizophrenia (46.67%), followed by paranoid disorder (26.67%), personality disorder (16.66%), and mood disorder (10%). A history of domestic violence was found in 40% of the cases and premeditation in 30% of the cases with two precipitating factors: a separation (real or fantasized) or a couples argument. The murder was most often perpetrated in the marital home, involved stabbing, and had no dissimulation of the wife's body. An alert had been raised by 20% of the victims before the murder. The analysis distinguishes two subgroups of motivational profiles. In the first group (73.33% of the cases), spousal murder occurred in a delusional context that could be favored by a break in psychotic medication or drug abuse. In the second group (20%), uxoricide happened in a dynamic of homicide-suicide.

**Conclusion:** The results of the present study on 30 mentally ill men who committed uxoricide were consistent with the literature on sociodemographic and forensic data, but not on clinical data. This study identified two psychopathological subgroups: a delusional context or a homicide-suicide dynamic. Biases in this study do not make it possible to provide a full generalization of those findings. Further studies are needed to better characterize this type of act.

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Uxoricide, Mental Illness, Forensic Psychiatry

## I26 Dr. Jekyll and Mr. Hyde—Child Pornography, Cybercrime, and Pediatrics: A Case Report From Argentina

*Maria S. Ciruzzi, PhD\*, Hospital Nacional de Pediatría Prof. Dr. Garrahan, Buenos Aires 1045, ARGENTINA*

**Learning Overview:** After attending this presentation, attendees will confront the challenges that arise at a pediatric setting when a sexual perpetrator is found to be part of the health team. Attendees will also understand the importance of local, regional, and international collaboration to discover, try, prosecute, and sentence this horrific crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing key aspects of the important roles that law, ethics, technology, and psychiatry are called upon to perform to protect children and patients. This presentation will also call attention to how best to prevent child abuse, protect pediatric patients, assure the best standard of care, and select the best professionals and best people to serve, which is a great dilemma that this shocking case puts at odds.

Child sexual abuse imagery and exploitation on the internet is an urgent problem. Last year in the United States, nearly 70 million images and videos of child sexual abuse, commonly known as child pornography, were reported to the authorities.

In 2018, technology companies reported over 45 million online photos and videos of children being sexually abused, more than double what they found the previous year. In 1998, there were over 3,000 reports of child sexual abuse imagery. Just over a decade later, yearly reports soared past 100,000. In 2014, that number surpassed one million. In 2018, there were 18.4 million; more than one-third of the total ever reported. Those reports included over 45 million images and videos flagged as child sexual abuse. That number originates almost entirely with technology companies based in the United States.

Child pornography reveals a problem global in scope, as most of the images found were traced to other countries as well. While the material, commonly known as child pornography, predates the digital era, smart phone cameras, social media, and cloud storage have allowed the images to multiply at an alarming rate. Both recirculated and new images occupy all corners of the internet, including a range of platforms as diverse as Facebook® Messenger, Microsoft's® Bing® search engine, and others.

In a particularly disturbing trend, online groups are devoting themselves to sharing images of younger children and more extreme forms of abuse. The groups use encrypted technologies and the dark web, the vast underbelly of the internet, to teach pedophiles how to carry out the crimes and how to record and share images of the abuse worldwide. In some online forums, children are forced to hold up signs with the name of the group or other identifying information to prove the images are fresh.

Offenders can cover their tracks by connecting to virtual private networks, which mask their locations, deploying encryption techniques, which can hide their messages and make their hard drives impenetrable, and posting on the dark web, which is inaccessible to conventional browsers. The problem of child sexual abuse imagery faces a particular hurdle. It gets scant attention because few people want to confront the enormity and horror of the content or they wrongly dismiss it as primarily teenagers sending inappropriate selfies. Common language about the abuse can also minimize the harm in people's minds. While the imagery is often defined as "child pornography" in criminal laws, experts prefer terms like child sexual abuse imagery or child exploitation material to underscore the seriousness of the crimes and to avoid conflating it with adult pornography, which is legal for people over 18 years of age.

This is the framework in which a case that involved a pediatrician in this criminal activity will be presented.

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### Child Abuse, Sexual Perpetrator, Psychopathy and Perversion

## 127 The Neurocognitive and Emotional Effects of Chronic Polychlorinated Biphenyls (PCB) Exposure

Pamela Mahoney, PhD\*, Valley Glen, CA 91401; Richard J. Perrillo, PhD\*, San Francisco, CA 94108; Jenny Brook, MS, Epidemiology Resources, Valley Glen, CA 91401; Erin Eloffson, MS, Epidemiology Resources, Valley Glen, CA 91401

**Learning Overview:** After attending this presentation, attendees will have an understanding of the prevalence of PCB exposure to polychlorinated biphenyls in United States schools, be able to identify the appropriate testing battery for patients with known or suspected chronic PCB exposure, and be able to recognize the characteristic pattern of neurocognitive and emotional deficits associated with PCB exposure.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness of the neurocognitive and emotional effects of PCBs.

**Background:** One of the more powerful indicators of any harmful chemical or toxic exposure is an understanding of the health effects that are caused by that chemical.<sup>1-3</sup> An estimated 12,900–25,900 schools in the United States are contaminated with PCBs.<sup>4</sup> Neurocognitive and emotional effects are among the most well-documented health effects of PCBs. This is a neurocognitive study of a group of 29 adults and 16 children who were chronically exposed to PCBs at school. Many of the occupants became ill. A lawsuit was filed and data collection ensued, including neurocognitive testing.

**Methods:** Knowledge of the lawsuit was by word of mouth. Participation was open to anyone who spent any time in the buildings. The present study consists of three age groups: young children ages 7–15 ( $N=12$ ); adolescents ages 16–19 ( $N=5$ ); and adults ages 21–58 ( $N=29$ ). Comprehensive neuropsychological testing covered seven cognitive domains: cognitive proficiency, reaction time, multiple aspects of attention, multiple aspects of executive function, auditory verbal learning and memory, visual memory, and fine and gross motor. Baseline premorbid Intelligence Quotient (IQ) was established with the Test of Premorbid Functioning (TOPF), used to predict pre-injury IQ and memory abilities. The TOPF was used for those above 20 year of age. For ages 16–19, the TOPF with parents' demographics only was used. For ages 7 through 15, the General Ability Index from the Wechsler Intelligence Scale for Children® Fifth Edition (WISC®-V) was used to predict a premorbid IQ. Automated Neuropsychological Assessment Metrics (ANAM) was used to establish cognitive proficiency across the cognitive domains as well as different aspects of reaction times. Prefrontal/frontal brain-mediated executive functioning was assessed using: Stoop Color Word Test, Go/No Go Task, Connors Continuous Performance Test (CPT), Trails Making Test (TMT), Wisconsin Card Sorting Task (WCST), Right/Left Orientation, and the Controlled Oral Word Association Test (COWAT). Auditory verbal learning and memory were assessed using the California Verbal Learning Test (CVLT 3) and the Episodic Memory component of the Wechsler Memory Scale®, Fourth Edition (WMS®-IV). Visual memory was tested using Rey Complex Figure Test (Rey CFT), Visual Incidental Learning, delayed recall from the ANAM, as well as the Visual Spatial Memory. Fine and gross motor functioning were measured using the Finger Tapping Test and Grip Test.

**Results:** Across all groups, multiple areas of impairments were evidenced in overall cognitive proficiency and reaction times, selective attention, sustained attention, divided attention, perceptual reasoning, single trial learning, visual memory and processing, and fine motor ability. In addition to cognitive impairments and decline and inconsistency with baseline predictions, anxiety (Post-Traumatic Stress Disorder [PTSD]), depression, irritability, and emotional distress were common findings.

**Conclusions:** Even low doses of chronic PCB exposure have measurable and sometimes dramatic neurocognitive and emotional effects that permeate the individual's life. The pattern of results clearly indicates neurocognitive issues significantly below baseline predictions and heightened emotional issues as a direct result of prolonged and chronic exposure to PCBs. These mostly genetic strangers have suffered and continue to suffer from these effects as damage, especially to adults' nervous systems, is rarely repairable.

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### Neurocognitive Testing, Polychlorinated Biphenyls (PCBs), Toxic Exposure

## I28 A Model for Mapping the Neural Circuits Underlying Impulsive Aggression

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**Learning Overview:** After attending this presentation, attendees will understand some of the major neural circuits underlying impulsive aggression through the comparison of the mechanisms of actions of three different Anti-Impulsive Aggressive Agents (AIAAs) of different classes: fluoxetine, phenytoin, and valproate.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a review of the mechanisms of action of three of the most studied agents used to manage impulsive aggression, as well as an outline of the proposed neurobiology of impulsive aggression. Discussion of the underlying mechanisms of action in the diverse range of medications that mitigate impulsive aggression provides a useful paradigm for clinicians and forensic evaluators managing a population with anger control problems, as well as consideration for future research.

Currently, there are no Food and Drug Administration (FDA) -approved medications with indications for treatment of impulsive aggression or intermittent explosive disorder. However, symptoms related to this condition significantly impact quality of life for patients and their families. The paucity of approved treatment options establishes an urgency for the development of effective therapeutic modalities. Three of the most widely used AIAAs are classified based on heterogeneous applications: Selective Serotonin Reuptake Inhibitor (SSRI) antidepressant (fluoxetine), anticonvulsant (phenytoin), and anticonvulsant and mood stabilizer (valproate/divalproex).<sup>1</sup>

An emerging model for impulsive aggression is the “top-down/bottom-up” hypothesis, where top down refers to the controlling neurotransmitters such as serotonin in the frontal lobes and Gamma-Aminobutyric Acid (GABA) and bottom up corresponds to excitatory neurotransmitters, specifically glutamate.<sup>2</sup> According to this hypothesis, an imbalance between control and excitation can lead to poorly controlled aggression. This can be the result of too little control from the prefrontal cortex or too much excitation from the amygdala. The role of fluoxetine in diminishing impulsive aggression is believed to be, in part, due to serotonin’s effect in enhancing prefrontally mediated self-control.

Impulsive aggression also represents an imbalance of the glutamatergic and GABAergic activity in the amygdala-hypothalamus-periaqueductal gray circuits. In line with the top down/bottom up hypothesis, and particularly the GABA-glutamate balance, there is evidence that valproate enhances GABA functioning by several direct and indirect mechanisms.<sup>3-5</sup> Valproate has also been shown to decrease glutamate activity by mainly indirect mechanisms, although the effects are less clear and the evidence less consistent across species.<sup>6,7</sup>

Because both seizure disorders and impulsive aggression are disorders of dysregulated neuroexcitement, it is conceivable that explanations of phenytoin’s anticonvulsant action may pertain to its therapeutic effect on impulsive aggression as well. In addition to limiting neuronal firing, phenytoin has been shown to increase GABAergic activity and decrease glutamatergic activity, which was found to be responsible for its anticonvulsant efficacy.<sup>8-10</sup> Given the evidence for GABA/glutamate imbalance as a neural mechanism for aggressive behavior, these findings could contribute toward understanding phenytoin’s mechanisms for reducing impulsive aggression.

Through review of current off-label treatment modalities, this study overviews the potential biochemical pathways that may be involved in impulsive aggression and could be potential targets for further research and development.

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### Impulsive Aggression, Anti-Impulsive Aggressive Agent, Glutamate

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## I29 Tele-Testimony to Overcome Testimonial Hearsay in Civil Commitment Hearings

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the evidence required for civil commitment in California; (2) the problems caused by a recent California Supreme Court ruling on hearsay evidence on a variety of legal issues; and (3) how tele-testimony can provide a solution to some of these problems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness about a solution to the challenge of testimonial hearsay, which extends beyond mental health cases.

The civil commitment (conservatorship) process in California requires a testifying psychiatrist or psychologist to offer proof beyond a reasonable doubt that the proposed conservatee, as a result of mental disorder, is “gravely disabled.” Grave disability is defined as a person being “unable to provide for his or her basic personal needs for food, clothing, or shelter.”<sup>1</sup> Generally, the testifying expert not only evaluates the patient, but also considers sources such as: hospital records, observations made by other team members such as nurses and recreation therapists, and discussions with outpatient providers, outreach teams, and family members. These sources provide information that the expert could not directly observe, such as the patient’s behaviors outside of a locked hospital unit.

In the California landmark case, *People v. Sanchez*, a gang expert used collateral information to opine that Sanchez was a gang member that led to the finding of gang enhancements on multiple felonies.<sup>2</sup> Sanchez appealed, contending the expert’s testimony was hearsay. The California Supreme Court reversed his gang enhancement charges holding that “when any expert relates to the jury case-specific out-of-court statements and treats the contents of those statements as true and accurate to support the expert’s opinion, the statements are hearsay (p.24).”

As testifying mental health experts generally use significant amounts of hearsay data in forming their opinion, the inadmissibility of such data after *Sanchez* rendered proving grave disability beyond a reasonable doubt extremely difficult to overcome. Collateral sources such as nurses, outpatient providers, and treatment staff from prior hospitalizations would have to testify physically at court for their testimony to be admitted, which places significant burdens on clinical staffing. Many facilities contract testimony to outside psychiatrists/psychologists, allowing the treating clinician to remain in the facility and continue to provide care. Typically, these outside psychiatrists/psychologists evaluate the patient once and use collateral information primarily when testifying. Following the *Sanchez* decision, without the use of collateral data, the evaluator may be especially challenged in that sufficient proof of grave disability is rarely obtained from one interview.

Tele-testimony (testifying remotely by videoconferencing or telephone) offers a practical solution to the testimonial hearsay challenges resulting from *Sanchez* and similar cases across the county. For the conservatorship issue, if the treating psychiatrist or psychologist does not have sufficient evidence of grave disability from their own observations, then clinical support staff can provide collateral testimony remotely without being burdened by traveling to and waiting at the court house for their case to be called.<sup>3</sup> Thus, they can remain at their facility providing care, freeing the facility from having to find covering staff. Moreover, the treating clinician can also testify in the same manner, obviating the need for contracting outside evaluators. Additional collateral sources such as family, teachers, and employers can testify without the inconveniences of going to court.

Currently, jurisdictions have varying rules regarding tele-testimony. In *United States v. Gigante*, the Second Circuit allowed video testimony from a witness in the Federal Witness Protection Program because he was fatally ill.<sup>4,5</sup> However, in *United States v. Yates*, the Eleventh Circuit denied the use of video testimony from witnesses in Australia who were only willing to testify via video.<sup>6</sup> The court stated that alternative forms of testimony can be used only “to further an important public policy.”

In 2018, the Los Angeles County Department of Mental Health proposed integrating tele-testimony into the Mental Health Court to alleviate the backlog of grave disability conservatorship cases.<sup>7</sup> Despite technological infrastructure already existing and used for specific cases (primarily state hospital patients, often hundreds of miles away from the court), prior to the COVID-19 pandemic of 2020, tele-testimony for local conservatorship cases was still not performed. Since then the pandemic has required the use of tele-testimony for some local cases such as patients who are COVID-19 positive.

In light of *Sanchez* and health limitations caused by the COVID-19, pandemic courts throughout the country are contending with how to admit testimonial evidence. Video and telephone services have been found to be a useful adjunct or replacement in non-forensic settings such as in medical care. Tele-testimony can be valuable in ensuring necessary information be admitted so that the court can make the most informed decisions while adhering to the hearsay rule.

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### Hearsay, Tele-Testimony, Mental Health Issues

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## I30 Telehealth Forensic Mental Health Evaluations: Benefits, Pitfalls, and Lessons Beyond the Pandemic

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the current state of telehealth treatment and practice parallels relevant to forensic psychiatric/psychological evaluations; (2) possible objections to telehealth forensic evaluations from evaluators, evaluatees, attorneys, and judges; and (3) benefits and risks associated with expansion of telehealth forensic evaluations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic mental health community by: (1) raising awareness of factors important in evaluating or considering to evaluate individuals via telehealth and videoconferencing technology; (2) highlighting best practices for evaluation; and (3) discussing the policy implications of forensic telehealth expansion.

There has been a significant expansion of telepsychiatry and telemedicine for treatment purposes as a direct result of the COVID-19 pandemic. Use of telehealth for forensic mental health evaluations has also occurred, but it is unclear to what extent this has increased.<sup>1</sup> It is likely that elements of this practice will persist beyond the pandemic, impacting how forensic evaluations are conducted.

There have been a few studies on administering competency to stand trial measures using videoconferencing, but there is insufficient research to reach conclusions about the reliability and validity of telehealth forensic psychiatric/psychological evaluations in general.<sup>2</sup> Research on clinical telepsychiatry shows that videoconferencing presents a variety of potential barriers. Most significantly, technological problems may reduce the quality of or interrupt entirely the video or audio elements. Even under ideal conditions, videoconferencing as a method of evaluation can limit the evaluator's ability to appreciate or detect clinically important observable clinical data.<sup>3</sup> Generally there is more difficulty establishing rapport (a key factor in an evaluator's ability to elicit information), especially when there is a lack of familiarity with the use of videoconferencing by either party.

If third parties are required to assist with the call, this too may limit rapport and willingness to disclose information. Evaluatees, particularly those who are unfamiliar with videoconferencing technology, may have a preconception of telehealth as being substandard or inferior to in-person encounters. Moreover, evaluatees may have concerns about the confidentiality and security of internet technology. Individuals who have serious mental impairments may also have difficulty using this medium.

Although the courts have increased the use of videoconferencing technologies for legal proceedings and even expert testimony with little objection, there has been little if any statutory or case law on telehealth for forensic mental health evaluations.<sup>2</sup> Attorneys and judges may question the validity and accuracy of the videoconferencing evaluation based on any of the previously described concerns. Additionally, evaluator competence and experience in conducting evaluations via video technology itself may be scrutinized. Even if videoconferencing evaluations are accepted, findings and conclusions must acknowledge known limitations of the telehealth medium in general. Due to these factors, telehealth evaluations in place of in-person evaluations could be challenged as a violation of due process. Other problematic issues may be raised, including recording and storing the telehealth evaluations.

In light of the accelerated use of telehealth for treatment purposes, the continued adoption of this technology for forensic mental health evaluation requires careful review and consideration. This presentation will provide a thorough discussion of currently known clinical, practical, and legal concerns, and highlight the need for carefully establishing principles, procedures, and safeguards.

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### Telehealth, Forensic Evaluation, Ethics and Policy

## J1 The Accuracy and Reliability of Forensic Handwriting Examiner Decisions

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**Learning Overview:** After attending this presentation, attendees will have learned about the results of the large-scale Handwriting Decision Analysis (Black Box) Study, including accuracy, error rate(s), reproducibility, and repeatability of handwriting decisions, and what factors impact these rates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling forensic document examiners to answer questions regarding what factors impact the accuracy and reproducibility of the decisions, including those related to the examiner and those related to the samples.

This Handwriting Decision Analysis (also known as Black Box) Study was designed and conducted by the Federal Bureau of Investigation (FBI) Laboratory, Ideal Innovations, and Noblis. The design of this study was based on that of the FBI Laboratory-Noblis latent print examiner black box study in 2011, and in consideration of comments made in two United States Reports: the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Pathway Forward*, and the 2016 Report from the President's Council of Advisors on Science & Technology titled *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*.<sup>1-3</sup>

Participation for this study was limited to practicing handwriting examiners who had performed casework within the past two years. A pre-test survey was completed by each participant to gather information about their education, training, experience, certification, and day-to-day work. Participants were asked to perform a total of 100 comparisons of paired handwriting samples, each containing one sample of questioned writing and up to five pages of writing from a single known subject. In an effort to represent the wide range of evidence encountered in casework, a diversity of handwriting samples was selected. The test samples included a range of styles of handwriting (i.e., cursive, hand printing, and mixed), content (e.g., addresses, a few sentences, predefined London or Center of Excellence for Document Analysis and Recognition (CEDAR) letters, or freeform text), known writing samples containing the same or different wording as well as varying amounts of questioned and known writing. Note that this study was limited to the comparison of handwritten documents and did not include the comparisons of signatures. Great effort was taken to provide participants with test packets that were relatively challenging, both for mated and non-mated pairs.

Using an online platform, participants were provided with high-resolution digital images that could be downloaded for comparison. The participants used this platform to respond to predefined questions that included the conclusion, limitation(s), assessments of comparability and variation within questioned and known writing samples, perceived difficulty of the comparison, and most influential characteristics that were used in making their decisions. Participants were given five conclusions to choose from, including definitive opinions (written by and not written by), qualified opinions (probably written by and probably not written by), and no conclusion. The study resulted in 7,213 trials from 86 handwriting examiners on 180 distinct comparisons of questioned and known handwriting.

This presentation will provide the results of the study, including the accuracy, reproducibility (inter-examiner variation), and repeatability (intra-examiner variation) of decisions made by forensic handwriting examiners. Also presented will be the factors that did (or did not) impact the accuracy of the decisions in this study, including those related to the examiner (such as training or experience) and those related to the samples (such as quantity of writing, comparability of content, limitations, or style of writing).

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### Handwriting, Error Rates, Black Box Study



## J2 An Empirical Exploration of Handwriting Theory With Regard to Natural Variation

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of natural variation in signatures and the methods used to define a writer's range of variation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information and new methods to quantify a writer's range of variation. The results of this study also provide statistical validation for the premise of natural variation.

Forensic handwriting identification is based on two universally accepted premises: no two people share the same combination of class and individual characteristics and no one person writes in the exact same way twice, which is referred to as natural variation. Statistical research has been conducted to validate the premise of individuality, but there is little published research to validate the premise of natural variation.

Slight differences or deviations found among repeated specimens of an individual's handwriting are referred to as natural variation and are expected in every person's writing. Variation is evaluated and determined by the Forensic Document Examiner (FDE) based upon training and experience and is used to decide if a new feature falls within a writer's range of variation. Some writers have a very small range of variation, seldom deviating from their habitual letter formations, spacing, relative heights, and speed. Conversely, others may possess a much wider range of variation incorporating numerous letter formations and features within their writing. Proper evaluation of variation is critical in an examination, as misinterpretation of a writer's range of variation may result in an erroneous conclusion. When little to no variation is present between two or more signatures, the genuineness or authenticity of the signatures is called into question.

The current study focused on 765 bank check signatures produced by a single writer over the course of 40 years. An American Board of Forensic Document Examiners (ABFDE) -certified forensic document examiner assessed the writer as possessing high skill and consistency. Manual comparison of the signatures to detect variation in handwriting features would be significantly time consuming, requiring more than 292,230 comparisons. Instead, a computational program was designed to conduct automated comparisons through pixel-by-pixel matching to quantify the percentage of overlap between each possible pairing of the 765 signatures. The signature comparison results provide preliminary data to support the premise that no one person writes the exact same way twice and strengthens the scientific basis for the validity of forensic handwriting examination. This study also demonstrates how a writer's range of variation can be measured and quantified.

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### Forensic Handwriting, Signatures, Variation





## J3 Measuring and Communicating Opinion Strength in Forensic Handwriting Examination: Concepts, Findings, and Future Directions

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**Learning Overview:** After attending this presentation, attendees will better understand reliability and validity in the context of developing opinion strength measures. Various opinion strength scales currently used by forensic handwriting examiners will be discussed, and findings from studies addressing opinion measures from research conducted by this study will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by teaching important components of the processes of empirically establishing the reliability and validity of measures of opinion strength and opinion language founded on the education, training, and experience of experts in the field.

The evaluation of the authenticity of writing is based on the premise that handwriting is a behavioral artifact that is distinctive because of features and characteristics within various forms of writing (e.g., signatures, hand printing, numerals). Both internal (endogenous) and external (exogenous) factors influence the appearance of writing. For example, a significant body of research demonstrates that a person's writing habit is the expression of endogenous physiological and neurological characteristics of the writer. Other research has demonstrated that exogenous factors such as the amount of space available for the writing or the angle of the writing surface result in different expressions of the writer's characteristic writing habits. These factors lead to the two primary tenets of handwriting examination: first, that no two people write exactly alike in all features and characteristics when considered cumulatively and in combination (*inter-writer* variation); and second, that no person writes exactly the same way twice (*intra-writer* variation).

A substantial portion of Forensic Document Examiner (FDE) training is devoted to the comparison of signatures, handwriting, and hand printing. During training, examiners learn methods of handwriting disguise and simulation, how to evaluate similarities and differences of writing, characteristics of distortions due to old age, illness, time pressure, and other methods of determining the quantity and quality of writing features observed during an examination. Examiners are trained to look for substantial similarities and differences among writing samples. They are also trained to look for repeated small characteristics that may be enough to establish clearly that writings are the work of different people, even though they may contain many general similarities. If two writings are by a single person, then no fundamental differences should exist.<sup>1</sup> A few fundamental, repeated differences may carry substantial weight in the examination outcome. During writing comparisons, examiners assign these similarities and differences some degree of evidentiary weight, which then forms the basis for the strength of the examiner's opinion.

This presentation addresses the findings of three studies investigating the reliability, validity, and measurement properties of measures of opinion strength currently used by handwriting experts in the United States and abroad. All studies used a multi-method approach in which professional handwriting examiners completed a series of comparisons of questioned and known handwritten signatures. In Study 1 and Study 2, they were then asked to give an opinion about whether they felt the questioned signatures were genuine or simulated in some way. After giving their opinions, examiners then gave their opinion strength using the nine-level American Society for Testing and Materials (ASTM) opinion measure. In Study 3, examiners viewed ten writing comparisons, including both signatures and extended writings. They were asked either to opine on whether the questioned writings were genuine or simulated or whether the two sets of writings were produced by the same writer or different writers. Then examiners were asked to give their opinion strength on each of seven different opinion measures currently in use in the United States and abroad. In Study 2 and Study 3, examiners were also asked to indicate their opinion strength on a sliding scale from 0 to 20 (Study 2) or 0 to 100 (Study 3).

Differences were observed in expressed opinion strength depending on whether the examiners felt that the signatures were genuine/written by the same person or simulated/written by different people. Another observation noted was that the boundaries between levels of opinion strength overlap, and that the range of opinion strengths varied according to the number of levels on the scale (3, 5, 7, 9). Examiners in Study 3 expressed that they were unclear in some instances as to how to apply opinion strength measure language to their decisions, suggesting that more research is needed to determine how the scale levels are understood both by examiners and by consumers of the examiner's opinions.

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### Opinion Measures, Reliability and Validity, Forensic Handwriting Examination



## **J4    Toward Consensus in Describing Handwriting**

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**WITHDRAWN**

## J5 Pen Pressure Measurement in Signatures of Women and Men Using 3D Digital Microscopes

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**Learning Overview:** After attending this presentation, attendees will be informed about an idea regarding the contributions of measuring pen pressure numerically with microscopes, measuring surface roughness, and future research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by indicating 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 such as measurement with 3D microscopes.

Frequently used diagnostic elements in signature examinations are pen pressure and pen pressure changes. Studies on pen pressure changes used to aid identification focus on online signatures placed on tablets.<sup>1-4</sup> However, the use of paper is still very common today.<sup>5</sup> The purpose of this study is to numerically measure the pen pressure depth using 3D digital microscopes and to investigate whether there is a significant difference between the pen pressure depths of the handwriting of men and women.

Ten female and ten male graduates of university and high school were asked to sign their signatures three times on three different surfaces, using the same brand of pen and paper of the same type. In the first case, signatures were signed on an A4-sized file placed on a “file with clamps;” in the second case, a blank A4-sized paper of the same kind was placed under the paper on which the signatures were signed; and in the third case, two blank files of the same type were placed under the paper on which the signatures were signed.

Images were taken at 300X magnification using a Leica® DVM-6 brand microscope at the points determined on the signature samples and their 3D profiles were created for analysis. At the determined points, markings were made on the two opposite sides of the line by means of the LAS X software integrated into the microscope used, and the numerical values and graphs were obtained by measuring the depth of 540 points in micrometers ( $\mu\text{m}$ ) at the distance between the two marker points. During the measurements, the maximum value given automatically by the software program was taken as the depth value at each point. In the study, it was investigated whether there is a difference between the depths of male and female subjects by using the independent sample *t*-test using the Statistical Package for the Social Sciences (SPSS) -25 software.

The values at each point of three signatures on three different surfaces were averaged for each person and, when it was investigated whether there was a significant difference between gender and signature depth using the independent sample *t*-test, it was found that there was no significant difference ( $p > 0.05$ ).

In signature examination and comparisons, it is extremely important to determine the depth left by the pen tip on the paper. Since individuals habitually do not press the pen at the same level, the depth of the trace left by the pen tip varies from person to person. It is very important to measure the depth of the trace left by the pen numerically. The use of a 3D microscope is an innovation in this study, while investigating whether there is a difference between the depths of the signatures of women and men. In the literature search, no study was found on depth measurement using the same method. Although the pen pressure depth is expected to be deeper due to the stronger physical structure of men, no significant difference was found between the pen pressure depths of men’s and women’s handwriting in this study. One of the reasons for this may be the small sample size, but it may also be based on the principle that the amount of force applied while performing an action is proportional to the work done, although the physical structure of men is stronger than women.

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### Signature Examinations, Pen Pressure, 3D Digital Microscopes



## J6 Tiger King—Forensic Document Evidence Involving the Disappearance of Jack Donald Lewis

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the documentary evidence that has been discussed during and subsequent to the airing of the Netflix® mini-series *Tiger King* and specifically involving the disappearance of Jack Donald Lewis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the detailed analysis of evidence often discussed in person and in the media concerning issues related to the mini-series *Tiger King*.

On August 18, 1997, Jack Donald Lewis left his home in Tampa, FL, to make an early morning delivery. He was never seen again. Lewis was a wealthy real estate and used car businessman with property holdings in Florida and Costa Rica. Lewis and his wife also co-founded Wildlife on Easy Street (now Big Cat Rescue). His estate at the time of was worth approximately five million dollars. Mr. Lewis' car was found on August 20 at the Pilot Country Airport some 40 miles away in Spring Hill. Mr. Lewis owned aircraft and was an experienced pilot. Investigations in Florida and Costa Rica purportedly turned up no evidence of foul play. With the release of the Netflix® mini-series *Tiger King*, authorities were hopeful that new evidence would come forward.

A questioned documents examiner was approached by two investigative reporters in order to conduct an examination of signatures on two documents. One was a Durable Family Power of Attorney (POA) dated November 21, 1996, and filed with Hillsborough County Clerk of Circuit Courts on September 2, 1997. This document had the unusual reference to its validity not being “affected by any disability or disappearance.” The other document at issue was a “Will and Testament” (Will) also dated November 21, 1996, and also filed with Hillsborough County Clerk of Circuit Courts on September 2, 1997. The work assignment was to determine whether or not the evidence supported that the signature of Jack Donald Lewis was written by Jack Donald Lewis.

An examination was conducted of the questioned documents and numerous documents bearing signatures of Jack Donald Lewis. During the assessment of the questioned documents, a step in the industry-wide standard process of examining handwritten items, it was noted that the Lewis signature entry on the POA and the two Lewis signatures on the Will looked nearly identical. Individuals have natural variation in their writing due to the inability to reproduce complex muscular movements with machine-like precision. There has never been a record of one writer producing two writings that are exact replications in every facet. However, in this instance, the signatures were nearly identical. The degree of similarity was striking. A review of the submitted known specimens noted normal range of variation in Mr. Lewis' writing such that a very unusual narrow range of variation could not be supported by the evidence.

A key piece of evidence submitted as part of the examination was a marriage record for Jack Donald Lewis and Carole Ann Stairs dated October 10, 1991, and filed with the Polk County, FL, Clerk of Circuit Courts on that same day. The provenance of this document was significant and was also a key factor in this examination as the Jack Donald Lewis signature entry on this document was also nearly identical to those on the POA and Will dated five years later. Both the POA and Will denote Carole Lewis as “wife.” As such, there is strong support that the marriage record pre-dated the questioned documents independent of the stated dates on the POA and Will.

Based on the evidence provided, it was opined that the Lewis signature entries on both the POA and the Will were tracings and that the signature entry on the marriage record was the model signature from which they were traced.

It was further noted that the notary signatures and witness signatures also bore nearly identical signature entries one from the other. While no identifiable model signature was located, it was opined that at least two of the three signatures on the POA and Will of each writer were products of tracing. Subsequent to this examination, the notary and one of the witnesses have disavowed their involvement in their alleged signature entries. The other witness has made no statement per this study's research.

While this material is interesting, particularly to those who watched *Tiger King*, it must be emphasized that this examination in no way proves or even implies anyone's direct or indirect involvement in the disappearance of Jack Donald Lewis.

The questioned documents examiner has not watched any episodes of the program.

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### Signature, Forgery, Estate

## J7 Anonymous Letters Examination: Where Forensic Linguistics Meets Handwriting Examination

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the contents and/or the way threatening letters are formulated when sent to the ruling party or an individual in power by their opponent.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a unique study in which written letters were analyzed regarding the scope of the threatening letter, the contents, sentence complexity and repetition of certain words, and formulations.

The aim of this paper is to examine the possibilities of profiling the author of the threatening letters by analyzing the utilized linguistic formulations and to cluster groups of anonymous texts under some potential common identity.

The assumption is that there is mostly one group of people who send politically driven threatening letters. Special attention should be given to the letters that are in some way "signed," which can then refer to the fact that the author is the same, but this does not necessarily have to be true. The importance of this research is that no one has dealt with this topic seriously in Croatia yet.

In the first phase, testing and analysis of the handwriting of a certain number of anonymous letters have been conducted in order to determine whether or not there is evidence that two pieces of handwriting have a common authorship (i.e., is there any evidence that two pieces of handwriting are written by the same person or not). Preliminary analysis was performed to check whether the collected anonymous letters met the professional standards of handwriting analysis 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 characteristics was performed in all anonymous letters individually. A stereomicroscope and video spectral comparator were used to compare handwriting samples, as well as capture images and measurements.<sup>1</sup>

Even though the scope of this research does not necessarily include the graphic analysis spectrum, the described preliminary analysis was necessary to perform the following forensic linguistic comparison of the achieved results. For the purpose of further research, the handwritten letters have been transferred into a digital format, partially for the purpose of performing a computer text analysis.<sup>2-4</sup> The initial result achieved by identifying the author based on the handwriting characteristics has been verified using the method of forensic linguistics.

After it was established that the author of all analyzed letters was the same, semi-automatic linguistic research of the letters was conducted in order to compare the results.<sup>5,6</sup> Based on the vocabulary used, word order in sentences, sentence complexity, used nouns, adjectives, and verbs, the intention was to determine whether the common text author could be established through such partially automatic linguistic analysis.<sup>7</sup>

Computational analysis showed that an average number of sentences in the letters was 13.538, with the shortest letter containing 9 sentences and the longest 25. When examining all the letters, an average number of tokens per sentence was 11.392. One letter contained an average of only 4.364 tokens per sentence, while the letter with the longest sentences contained an average of 19.280 tokens. The data was compared to two general corpora of Croatian language: the Croatian web corpus hrWac and the Croatian Language Corpus (CLC). hrWac (version 2.2) contains 1.3 billion tokens compiled from documents collected on the *hr* domain. In this corpus, an average sentence contains 17.952 tokens.<sup>8,9</sup> CLC is a corpus containing documents written in standard language and contains 100 million tokens. An average number of tokens per sentence in this corpus is 18.074. The computational analysis has shown that the subject sentences are shorter than the ones in hrWac and CLC by approximately six to seven words.

Finally, it should be emphasized that the present research is a case study. In order to draw a universal conclusion, the research should be conducted on a larger number of letters. An interesting aspect of the conducted research is the analysis of handwritten letters and the comparison with the ones obtained through a linguistic and computer-helped analysis of individual words, sentences, or a text.

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### Forensic Linguistics, Handwriting Examination, Computational Analysis

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## J8 Forensic Handwriting Analysis of Judahite Biblical Period Inscriptions

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**Learning Overview:** The goal of this presentation is to demonstrate the applicability and usefulness of the modern handwriting expertise methodology for the study of ancient inscriptions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that, per research, this is the first-of-its-kind handwriting analyses of 2,600-year-old archeological artifacts, which has a strong impact on the history knowledge and the literacy dissemination in the ancient world.

The paleo-Hebrew inscriptions from the Arad fort are one of a few textual corpora from the First Temple period. Dated to ca. 600 Before the Common Era (BCE), the eve of Nebuchadnezzar's destruction of Jerusalem, more than 100 ostraca (texts written in ink on clay potsherds) provide a record of distribution of provisions to military units shortly before the destruction of the Kingdom of Judah by the invading Babylonian army. Due to the site's isolation, small size, and texts that were written in a short time span, the Arad corpus holds important keys to understanding dissemination of literacy in Judah 2,600 years ago. These studies aimed at identifying the number of "hands" (distinct writers) in the Arad corpus. These encompassed 16 ostraca, but since two of them were double-sided, the number of texts analyzed was, in fact, 18. Per research, no such large-scale pair-wise assessments of ancient inscriptions by a forensic expert has previously been published.

The 18 inscriptions were selected because of their relative clarity and potential for character reconstruction. The original ostraca were examined in a number of museums and storage sites. When necessary, high-quality regular and/or multispectral images of the same ostraca were used.

The Paleo-Hebrew alphabet at the First Temple period is different from the modern Hebrew writing but preserves a number of basic similarities: it consists of 22 letters and is written from right to left with mostly separated characters.

The procedure followed the protocol of modern forensic handwriting examination and included a detailed examination of every single inscription according to the following features: general appearance of the sherd, writing style, arrangement and use of space, size and proportions, slant, punctuation, spacing, alignment of words and letters relative to an imaginary baseline, letter shapes and range of their variations within a script, and extraction of distinctive features. Consistent patterns and repetitions, common for different inscriptions, were identified: the same combinations of letters, words, punctuation, relative position of letters vis-à-vis the preceding and following letters, etc. Finally, an evaluation of identicalness or distinctiveness of various writers was made, based on the standard terminology guide for expressing conclusions of forensic document examiners. The grades range from the definite conclusion of identity to the definite elimination of identity. Inconclusive grade was used when there were significant limiting factors.

One encounters many limitations upon examining ancient inscriptions, such as rather poor state of preservation, fading and abrading of some ostraca, as well as flaking-off in places resulting in a partial loss of the ink; the texts are short and may not contain the full range of letters and their natural variations; limited knowledge about the writing instruments and their effect on the writing results; etc. These limitations were taken into account and were reflected in the conclusions. The highest grade of a positive identification was "strong probability that the two ostraca whose numbers 1 and 7 were written by the same writer." Regarding the negative degrees of certainty, it was easier to reach the higher degree of elimination since a sufficient amount of differences was found.

According to the results of the examination, 12 different "hands" were involved in composing 18 examined texts with varying degrees of certainty. That conclusion illuminates the issue of literacy in the Kingdom of Judah at the end of the First Temple period and suggests that many of the inhabitants were able to read and write, and that literacy was not reserved for a handful of royal scribes.

This study is a part of a broader interdisciplinary research carried out in cooperation with a team of the scientists (archaeologists, mathematicians, and artificial intelligence experts) at Tel Aviv University. The forensic handwriting examination was performed in parallel with the advanced machine learning algorithms analysis. Substantial agreement between these two independent methods was found. The findings of the combined study were published on September 9, 2020, in the journal *PLoS ONE*.<sup>1</sup>

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### Handwriting Analysis, Biblical Inscriptions, Arad Corpus



## J9 The Creation of a Demand Note Reference Collection

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**Learning Overview:** This presentation will explore using three different digital platforms, Adobe® Bridge, Microsoft® Office Excel®, and Pikaso Write-On, to create a searchable demand note reference collection. A demand note is a written request for the receiving individual to perform an action, often used in bank robberies, pharmacy robberies, or threats. The intent is to compare the features of each of these programs and evaluate how effective they are in helping make associations between demand notes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a general framework on how to create their own digital reference collection and tailor it to their specific needs.

The Bank Robbery Note File (BRNF) was first developed in 1985 by the Federal Bureau of Investigation's Document Section to provide a highly effective investigative service designed to assist with robberies where demand notes had been used. The basis for needing such a tool was that investigative experience demonstrated that subjects involved in the passing of such notes would continue to commit robberies until apprehended. Through the use of the BRNF, demand notes can be searched against each other in an attempt to make an association between cases. This can be of immense value because what may appear as an isolated bank robbery case may actually be a string of robberies that may never have been connected through conventional investigative techniques. Today, the BRNF contains more than 11,000 demand notes and uses the program Microsoft® Access to search and make associations with ease.

Currently, the Michigan State Police Questioned Document Unit has a total of 124 demand notes, spanning 20 years, with no defined program in place to assist in making associations between them. Looking to recreate the success of the BRNF at the state level, this study was focused on the development of a reference collection for demand notes encountered in casework. Three different digital platforms, Adobe® Bridge, Microsoft® Office Excel®, and Pikaso Write-On, were tested to evaluate the functionality and flexibility for a searchable demand note reference collection. Adobe® Bridge is a free digital asset management program that allows the user to preview, organize, edit, and publish files. An important feature of this program, and its draw for this study, is that a file's metadata can be edited. Specific keywords and labels can be assigned to a file, then searched using powerful filters and advanced metadata search features. Pikaso Write-On is a licensed program developed to collect, search, and manage large quantities of various documents. Many forensic document examiners in the field today are familiar with Write-On and its ability to assist in analyzing handwriting. Microsoft® Office Excel® is a licensed spreadsheet program that uses a grid of cells arranged in numbered rows and letter-named columns to organize data. Using several columns, features of each demand note were able to be documented and organized. In addition, a hyperlink can be associated to each demand note so that when clicked, the actual image can be visualized. In the end, a list of pros and cons from each of these programs was compiled and evaluated.

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### Questioned Documents, Digital, Reference Collection



## J10 The Forensic Document Examiner's Method of Communication in Court: The Chart

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**Learning Overview:** The goal of this presentation is to provide the history of forensic document examiner use of charts as a demonstrative tool during testimony.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing the importance of using charts to demonstrate the examination process and conclusions to clients and the trier-of-fact.

Forensic document examiners who have completed a formal two-year training program that meets the Scientific Working Group for Forensic Document Examination (SWGDOC) Standard for Minimum Training Requirements for Forensic Document Examiners are trained to conduct detailed examinations. Successful completion of formal training provides the examiner with the skill set to conduct examinations and reach a conclusion based on the individuality of the writing and the combination of characteristics. Based upon their training and expertise, forensic document examiners are considered expert witnesses who are allowed to present their findings in court.

Forensic document experts explain their findings to their client, to the judge, and to the jury, none of whom are knowledgeable in this field. Since the details of the examination process will be explained to laypersons, the examiner must use a method of communication that will assist the client and trier-of-fact to understand the process and the conclusion. One method of communication to achieve this goal is the use of demonstrative charts.

Forensic document examiners have relied on charts as tools to demonstrate their findings in court since the early 1900s. Albert Osborn, Ordway Hilton, and Wilson R. Harrison discussed the value of charts in their books.<sup>1-3</sup> In his treatise, *Questioned Documents*, Albert Osborn wrote it may be helpful to use a large chart with certain characters in the writing marked or highlighted.<sup>1</sup> Wilson R. Harrison stated in his treatise, *Suspect Documents*, that comparison charts comprised of enlarged text are of great value to facilitate an examination as well as for use during testimony.<sup>2</sup> In *Scientific Examination of Questioned Documents*, Ordway Hilton wrote photographic enlargements (charts) were essential to witness testimony.<sup>3</sup>

Laypersons tend to focus on gross or class characteristics and not the combination of individual characteristics in a writing or signature. The Kam studies dated between 1994 and 2001 revealed forensic document examiners consistently outperformed laypersons. These studies not only established that forensic document examiners who completed a two-year formal training program that complied with the SWGDOC Standard for Minimum Training Requirements for Forensic Document Examiners outperform laypersons, it highlighted the importance of the expert to dedicate time to educate the client or the trier-of-fact as to the examination process and the meaning of the issued conclusion. Charts provide a visual demonstration to assist a jury and judge in understanding the examination process.

This presentation will discuss how charts have changed from the early 1900s to 2020. Survey responses from forensic document examiners who described format, purpose, and the technology used in creating their charts will be discussed.

### Reference(s):

1. Harrison, Wilson R. *Suspect Documents Their Scientific Examination* (Nelson-Hall Co., Chicago 1958), 499.
2. Hilton, Ordway. *Scientific Examination of Questioned Documents Revised Edition* (Elsevier Scientific Publishers B.V., 1982), 395.
3. Osborn, Albert S. *Questioned Documents Second Edition* (Nelson-Hall Co., Chicago 1929), 267.

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### Chart, Demonstrative Exhibit, Technology





## J11 Standards for the Examination of Documents Using a Digital Workspace

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**Learning Overview:** The goal of this presentation is to explore published consensus standards in the field of forensic document examination to highlight procedures and considerations in these standards as they relate to conducting examinations of digital representations of test items. The intent is to show that although many of the standards were written with the examination of physical items in mind, the standards are equally applicable to digital representations and digital workflow processes for these same types of test items.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ensuring attendees will have a more comprehensive understanding of current Forensic Document Examination (FDE) standards and how they apply in a digital workspace.

Early forensic examination of documents relied on the physical examination of documents combined with photographic techniques. Some of these photographic techniques were efforts to more easily note and compare features. Photo enlargements provided more detail for gross comparisons; photomicrography illustrated finer details observed; and photographic superimposition gave the early FDE a method for overlaying and comparing test items that may share a common source. Other photographic techniques were used to conduct direct examinations of test items. This includes manipulating exposure and the use of alternate light sources with different barrier filters to reveal differing compositions of inks or substrates, and using transmitted light sources for the comparison of various substrates. These photographic techniques were eventually supplanted by the use of photocopiers, then scanners for some tasks and digital photography for other tasks previously accomplished by traditional “wet” photography. While the method of image capture has changed, many of the current standards were written with the traditional physical comparison methodology in mind (e.g., printing digital images to make comparisons). In the traditional methodology, the FDE examines the test items and makes notes of the observations. Even though the traditional physical model was the practice during the writing of many of the current forensic document examination consensus standards, they can equally be applied to a digital comparison workflow.

In a digital comparison workflow, many of the tasks previously accomplished by physical examination and/or photography can be easily completed with digital representations of the test items. An example of this is examination and association of writing indentations in paper. In the traditional workflow, the FDE examines the document with oblique lighting and/or processes the document with an Electrostatic Detection Device (EDD) and makes a physical transparency lift. The results are then physically compared to the suspected source writing and the FDE notes the common characteristics to determine source attribution. In a digital workflow, the same oblique lighting and EDD examination are conducted; however, it is only a digital image of the results that are compared to digital images of the suspected source of the impressions.

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**Standards, Digital, FDE**

## J12 The Use of Digitally Captured Signature (DCS) Technology in Everyday Casework

*Niko Kalantzis, MSc\*, Charotularios PC, Piraeus, Attiki 18535, GREECE*

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**Learning Overview:** The goal of this presentation is to familiarize attendees with methods of using and incorporating DCS technology in everyday casework in a laboratory environment, either for analysis, presentation, or sample collection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing comprehension and adaptation of the new technology to routine casework.

Forensic Handwriting Examination (FHE) research in DCS usually focuses either on the examination procedure of a disputed DCS or the comparison of signing behavior between glass and paper. Consequently, many examiners believe that they need to deal with DCS technology only when they encounter a case with a disputed DCS signature.

Yet there is another aspect of DCS technology that can enhance the FHE analysis in everyday casework that focuses on traditional Pen and Paper (P&P) signatures. The different options of representation of the captured information of DCS signatures allow the experts (through the use of specialized programs) to visualize different aspects of a signature formation.

This presentation focuses on the benefits of using DCS technology to collect comparison samples for P&P cases, the process of sample collection (either for a disputed DCS or a disputed P&P case) using several different hardware options, as well as the benefits of using the Wacom® Clipboard (PHU-111) for the DCS capturing, highlighting the managerial aspects of the process.

The Wacom® Clipboard allows simultaneous capturing of a signature formation in both A4 paper form and digitally, enhancing the managerial aspects of the process within casework with respect to document creation and handling. The simultaneous creation of a paper version and a full digital version of a document (in PDF) resolves a lot of legal aspects of report writing and record keeping within an organized laboratory environment while at the same time giving the expert the opportunity to collect and analyze DCS data.

Finally, some examples of using collected DCS samples in real cases will be presented to highlight the advantages of such practices in routine cases where the disputed signature has been executed on paper with a pen.

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### **Bioimetric, DCS, Signatures**



## J13 Electronic Signatures: What Are They, What Do We Know About Them, and What Do I Do When an Electronic Signature Case Lands on My Desk?

*Kathleen Annunziata Nicolaidis, BA\*, Associated Forensic Laboratory, LLC, Phoenix, AZ 85013*

**Learning Overview:** After attending this presentation, attendees will be more knowledgeable on research conducted in this field, the different types of electronic signatures, and what to do when contacted about an electronic signature case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating forensic document examiners about electronic signatures, what questions to ask submitters of electronic signature cases, and what tools are available for working these types of cases.

More and more, our signatures are being collected electronically. We are signing with our fingertips on phones or tablets, with a stylus on a signature pad, or by simply clicking a mouse button. Forensic document examiners around the world are encountering questions and cases involving electronic signatures. Attendees will learn about the different types of electronic signatures, research conducted in the field, and what to do when contacted about an electronic signature case. Results of a survey will be shared that will assess the prevalence of electronic signature cases around the world.

Although the United States Congress passed the Electronic Signatures in Global and National Commerce Act (ESIGN) in 2000, confusion remains about electronic signatures over two decades later. There are different types of electronic signatures, a multitude of vendors supplying software and hardware to businesses and governments incorporating electronic signatures into their workflow, and various regulations regarding their implementation. For instance, internet research unearthed a website providing Frequently Asked questions (FAQs) on electronic signature compliance with the Federal Drug Administration (FDA).<sup>1</sup> Below are just a few of the questions answered on the site:

- What methods may be used to create valid electronic signatures? **There are no mandated methods and part 11 regulations permit a wide variety of methods (from 2017).**<sup>1</sup>
- Does signing a true signature on a tablet comply with regulations? **It can. The basic approach of linking a subject-applied signature to an electronic consent form using a computer tablet's stylus feature can be considered part 11 compliant if consistently used correctly as intended (from 2014).**<sup>1</sup>
- What requirements must electronic signatures based on biometrics meet to be considered an accepted biometric method? **Electronic signatures based on biometrics must be designed to ensure that they cannot be used by anyone other than their genuine owners. They are accepted as long as they meet the requirements in part 11 (from 2017).**<sup>1</sup>
- Can an image copy of a signature be used instead of hand signing documents? **No. This is not considered an eSignature, and it provides no measure of security or assurance that the signatory actually drafted/reviewed/approved the document (from 2012).**<sup>1</sup>
- Is it an acceptable approach for a user to sign in to a secure data management system with their ID and password, and then only require the password to execute eSignatures during that log-in session? **Yes, this should be Part 11 compliant (from 2013).**<sup>1</sup>

The summaries presented in this library are for informational purposes only, they are not for implementation in operations. Please consult official Food and Drug Administration (FDA) guidance documents for operational use.

Forensic document examiners should not only be aware of the different types of electronic signatures, but also of the research conducted to determine if authorship of electronic signatures can be determined. An outline of research conducted both in the United States and abroad will be presented. Finally, discussion will be presented on the questions to ask of persons submitting electronic signature cases, the evidence needed, and the tools available to conduct the exam. Results of a survey will be presented discussing the prevalence of electronic signature cases around the world.

### Reference(s):

- <sup>1</sup>. <https://florencehc.com/library-fda-esource-eregulatory-guidance/signatures/>. Accessed 10/22/20.

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### Electronic Signatures, Casework, Questioned Documents

## J14 Normalization and Comparability of Digitally Captured Signatures (DCS)

*Niko Kalantzis, MSc\*, Charotularios PC, Piraeus, Attiki 18535, GREECE*

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**Learning Overview:** The goal of this presentation is to introduce attendees to the problem of comparability and the necessity for normalization of DCS from different sources. Furthermore, the necessary requirements to achieve such comparability will be discussed, and, finally, the methodology to achieve normalization will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by familiarizing attendees with the problems of different DCS sources to equip them with the knowledge of how to apply the normalization methodology and achieve comparability of DCS from different sources.

When it comes to signatures, the shift from pen and paper to digital media is attainable but not trivial. In the physical world, different writing instruments of the same class form the executed signature in the same manner, but this cannot be said for biometric signatures (also known as DCS) when captured with different software and hardware solutions (or even using the same hardware but different software only). This issue must be dealt with as DCS are increasingly used worldwide to replace pen and paper signatures, and both national and international legislation recognizes their equivalence as legally binding biometric data.

This study addresses two aspects of this problem. The first part of the study involves the comparison of 1.200 samples created with an XY-plotter (AxiDraw® v.3), which were collected from six identical digitizer pads (Wacom® STU-530) and the same software suite (Namirial® FirmaCerta Forensic) with different combinations of the six default styli provided with the pads and four inking pens (Wacom® Bamboo Spark). The comparison of the collected data from the same repeatable stimulus discusses the repeatability and the accuracy of the recorded stimuli from the Wacom® STU-530 digitizer. The second part of the study involved the analysis of the extracted (unnormalized) Comma-Separated Values (CSV) data from different software and hardware solutions and the construction of a normalizing procedure for those to be accurately analyzed under the same DCS software analysis suite. Through the analysis of the actual method of recording the biometric channels (X, Y, F, and T), the construction of a translation spreadsheet allows the accurate rearrangement of the collected data so that they can be read and analyzed in the International Organization for Standardization (ISO) 19794-7:2014 compliant CSV format of Namirial's® FirmaCerta Forensic DCS signature analysis software.

Finally, as a result of the study, the necessity to calculate the exercised force/pressure-level correlation function (zeta function) is recognized, and the calculation process of that function and its inverse is presented with practical examples that lead to DCS normalization and therefore comparability.

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### DCS, Biometric, Signatures

## J15 In-Air Trajectories (IATs): Applications of an Emerging Handwriting Characteristic

*Niko Kalantzis, MSc\*, Charotularios PC, Piraeus, Attiki 18535, GREECE; Michael Pertsinakis\*, Chartoularios, Piraeus 18535, GREECE*

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**Learning Overview:** The goals of this presentation are to introduce attendees to Electromagnetic Resonance (EMR) technology and the capabilities of the application of such technology to digitally captured signatures and to comprehension of the analysis and research capabilities of such technology to the area of forensic handwriting examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by familiarizing attendees with the use and capabilities of EMR technology.

The development and incorporation of digitally captured signatures (also known as biometric signatures) in routine business transactions worldwide has led to the advancement of the technology used in that process. As a result, several new features (either entirely new or old but represented in a new quantitative way) are now available for the forensic expert to analyze. One of these features relies on the capabilities of EMR technology that allows for a digitizer pad to locate the position of the tip of the compatible stylus without the need for actual contact between the two and hence map the trajectory of the stylus while moving in the air.

IATs—also known as in-air movements—are the invisible movements that are executed when the pen is lifted above the writing surface. These movements can be recorded using an electronic capturing device (e.g., a signature pad) of appropriate technology (i.e., EMR). The aim of this presentation is to demonstrate the potential of IAT analysis in forensic and non-forensic areas.

In the forensic field, research suggests that IATs are individualized characteristics that differ between genuine and forged signatures, with an increase in duration, jerk, and absolute size in the latter case. In the medical field, findings suggest an association between changes in IATs and different pathologies such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and depression, among others. Non-forensic research also supports the hypothesis that IATs are a part of the open loop motor program, as they are not influenced by writing systems or visual feedback. In conclusion, this study proposes the analysis of IATs along with on-surface trajectories in digitally captured signatures and reinforces the need to develop adequate methodology for IAT analysis. Further research should also be conducted to verify the repeatability of IATs, their identifying value, and to determine whether they are redundant.

Finally, additional information before and after the execution of a signature formation—that are now available for analysis with the proper software/hardware combination—will be discussed.

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### Biometric, In-Air, Signatures

## J16 A Best Practice Manual for Forensic Examination of Digitally Captured Signatures (DCS)

*Tomasz Dziedzic, PhD\*, Institute of Forensic Research, Krakow, POLAND; Niko Kalantzis, MSc\*, Charotularios PC, Piraeus, Attiki 18535, GREECE*

**Learning Overview:** The goal of this presentation is to introduce attendees to the problem of the examination of authenticity of DCS with respect to the methodology for traditional pen and paper signatures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by ensuring attendees will be familiarized with the product of the research project (i.e., the additional appendix to the Best Practice Manual of European Network of Forensic Handwriting Experts [ENFHFX] that deals with DCS analysis) so attendees will have a compass to address the issue.

Recent years have seen the increasing use of DCS (also known as biometric or dynamic signatures) for the authentication of electronic documents (e.g., Portable Document Formats [PDFs]). There have also been the first cases reported of forensic examination of such signatures commissioned by judiciary bodies. However, examination of DCS differs from that of conventional (pen-and-paper) signatures, not only on the traditionally examined characteristics that derive from the dimensional measurements, but also on the recording and examination of the dynamic characteristics of signature formation—as DCS technology allows capturing of both time and force on the Z-axis—marking a shift from qualitative to quantitative analysis.

Therefore, the ENFHFX, a Working Group of the European Network of Forensic Science Institutes (ENFSI), found it necessary to define rules of good practice for forensic examination of DCS. In the years 2018–2020, a research project called STEFA G8 was carried out, which resulted in the development of a relevant appendix to the Best Practice Manual (BPM) for Forensic Examination of Handwriting, used by ENFHFX member laboratories since 2015.<sup>1</sup> In the document, new DCS-specific terminology was introduced, an overview procedure DCS analysis and comparison proposed, and the following subjects were discussed: division of tasks between Forensic Handwriting Examiners (FHE) and forensic IT, the notion of an original in digital evidence, as well as hardware- and software-related subjects.

In addition to the BPM, a list of requirements was produced that aimed to make the signature data as suitable to forensic examination as possible. These recommendations are directed to DCS-related hardware and software manufacturers and users as well as for legislative bodies that are responsible for legal regulations related to the subject.

Furthermore, experimental research was conducted to answer the following questions: (1) Is writing behavior any different between signing digitally and signing on paper, and therefore can conventional samples be used as reference material when a DCS is disputed, and vice versa? It was found out that despite statistically significant differences in particular features between DCS and pen-and-paper signatures observed, no general tendency of different signing behavior was noted, which could carry the risk of erroneous conclusions in casework. This led to the conclusion that comparing DCS and conventional samples is justified; and (2) Are there any differences in the structure of biometric data that were captured with different hardware and software combinations? The results indicated that such differences may indeed occur and, therefore, data may not be directly comparable. Moreover, different ways of coding certain signature features were detected. Therefore, before the forensic examination, it may be necessary to scale and normalize signature data. Measures are proposed to conduct such operations.

Apart from the tasks accomplished by the STEFA G8 Project Team, future plans and capabilities that even involve the creation of DCS-related databases will be discussed as they derive from the products of the aforementioned research.

### Reference(s):

<sup>1</sup> <https://enfsi.eu/docfile/best-practice-manual-for-the-forensic-examination-of-handwriting-version-02/>.

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### Handwriting Examination, Digitally Captured Signatures, Best Practice Manual



## J17 Document Abnormalities Related to Portable Document Format (PDF) Technology

*Joseph L. Parker, Sr., MSA\*, Atlanta Forensic Document, Peachtree City, GA 30269*

**Learning Overview:** The goal of this presentation is to increase Forensic Document Examiner (FDE) understanding of document content abnormalities that can occur through the use of PDF digital file technology.

**Impact on the Forensic Science Community:** Questions as to the origin and genuineness of disputed documents frequently require FDEs to consider the effects of PDF technology. This presentation will impact the forensic science community by providing samples to assist FDEs in increasing their understanding of PDF technology and some of the document content abnormalities that can occur through use of PDF digital file technology. Knowledge gained during this presentation should assist FDEs in recognizing and correctly identifying similar features they may observe in future examinations of machine-generated documents.

FDEs are frequently tasked with assessing the origin and/or genuineness of disputed machine-generated documents. While an FDE may correctly opine that a disputed signature is written by the same writer of submitted known signature samples, in some cases the FDE completely neglects their obligation as a forensic expert if they concurrently fail to assess evidence within the same document that demonstrates the signature was inserted as a digital image, thereby confirming the document to actually be a cut-and-paste production.

Adobe® Systems Inc. introduced PDF digital file technology in 1993. In July 2008, Adobe's® PDF version 1.7 became International Organization for Standardization (ISO) 32000-1, the Standard for Electronic Document Archiving. Members of ISO/TC 171/SC 2/WG 8 (working group) continued development of the specification, culminating in the publication of version ISO 32000-2 or PDF 2.0. Today, there are numerous applications/software resources involving PDF technology.

PDF digital file technology use has flourished since its introduction in 1993, becoming an integral aspect today of worldwide machine-generated document production in homes, schools, businesses, and government. The proliferation of PDF technology in document production logically requires the FDE to have a fundamental understanding of PDF technology and its potential effects on machine-generated documents.

Beginning in the mid-1990s, several examinations were conducted of disputed documents that contained feature abnormalities of texts, images, and other content. During the same period, many FDE colleagues were assisted, primarily in the United States, in identifying PDF technology tools and their functions as sources for the various feature content abnormalities within disputed documents.

Research to isolate PDF technology tools, their functions, and nuances of interactions with other software and hardware was conducted by recreating the abnormalities in the documents being examined. Practical testing for the research involved using "period materials" contemporary with the purported dates of respective document problems. For example, testing for documents dated 1996 was conducted with computers having operating systems no newer than Windows® 95, Microsoft® Office Word no newer than Word 1995, and Acrobat® PDF versions no newer than 3.0 (PDF 1.2), released in 1996, as well as office-machine printers manufactured no later than 1996.

This presentation will include examples of abnormalities observed within actual documents from FDE casework, source(s) for which were confirmed by practical testing. Brief descriptions will relate the respective feature/function interactions between application software, hardware, and PDF technology that resulted in the respective abnormalities within printed copies of respective machine-made documents, their associated PDF archive digital files, and examples of discrepancies between PDF digital files and the documents they purportedly contain.

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### PDF, Documents, Abnormalities



## J18 Inconspicuous Writing Features: An Illustrated Glossary

*Jacqueline A. Joseph, BA\*, J. Joseph & Associates, Portland, OR 97205*

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**Learning Overview:** After attending this presentation, attendees will have expanded their knowledge of inconspicuous writing features for use in forming and explaining a fact-based opinion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by lessening the chances of wrongful convictions by the effective use of inconspicuous features in forming and explaining a reliable opinion of writer identity in order to serve truth and justice for all.

Inconspicuous features play a pivotal role in forming an opinion because, subtle as they may be, to the trained eye the features are observable, measurable and, in some cases, habitual. As such, they link the writer to the written. By viewing the illustrations, attendees may expand their knowledge of inconspicuous writing features and may gain a better understanding of what to look for during the examination process that is significant for use in forming an opinion.

The glossary's select group of 12 inconspicuous features are: malformed letters due to impaired muscle tension and release; precise covering strokes on upper, lower and mid-zone letters; breaks in the progressive motor sequence; circle letter movement direction (clockwise/counter-clockwise); and movement direction of the entry and exit strokes on and off the paper.

Each illustration will be accompanied by a brief description. The descriptions become a critical tool for use in presenting and explaining the basis of the examiner's opinion. The illustrations, coupled with the descriptions, reveal the writer's unique movements as they pertain to writer identification (linking the writer to the written) of the evidence.

Attendees will learn the definition of an inconspicuous feature that includes three properties (observable, measurable, and habitual) and three additional factors (hidden from the writer's attention through his/her being unaware of writing it in that particular way, difficult movement for the writer to intentionally suppress or change, and difficult for an imitator to notice, let alone incorporate, while attempting to write a successful imitation).

As a result of learning the objectives of this presentation, the forensic examiner may: (1) gain wisdom: the developed ability to perceive inconspicuous features allows the examiner to build and defend a reliable opinion of authorship; (2) communicate clearly: the use of the descriptions allows the examiner, whether to a lay audience or to one's peers, to clearly explain the properties of the features used to form the opinion; (3) rebut effectively: the confidence to stand up to the challenges of cross-examination is significantly boosted when one can explain the opinion beyond simply relying on form-matching and/or common qualities alone; and (4) check reliability: to lessen the chances of being mistaken that may lead to a wrongful conviction, the effective use of inconspicuous features in forming and explaining a reliable opinion of writer identity serves truth and justice.

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### Fact-Based Opinion, Writer Identification, Inconspicuous Features



### J19 An Evaluation of Gray Value Measurements and Hyperspectral Imaging (HSI) as a Method for Differentiating Optical Characteristics of Porous-Tipped Pen Inks

*Clarra G. Moore, BS\**, Sam Houston State University, Huntsville, TX 77320-3907; *Patrick Buzzini, PhD*, Sam Houston State University, Huntsville, TX 77340

**Learning Overview:** After attending this presentation, attendees will have gained knowledge about the capabilities of objective image analysis using gray value measurements and HSI aiming to increase the differentiation between porous-tipped pen ink samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding on gray value measurement research and exploring HSI for the applicability of pen ink analysis to increase confidence and transparency in forensic document examinations.

Porous-tipped pens, commonly recognized by their absorbent porous tip composed of compressed felt, fiber, plastic, nylon, or ceramic, are an under-researched area in the field of questioned documents. Most research has been focusing on ballpoint and gel ink pens since these types of pens are often seen in casework. Porous-tipped pens appeared commercially in the 1950s and have since evolved into popular writing instruments for artists, designers, hobbyists, and journalists alike. These pens can be used to create pieces of art, comics, fashion or interior designs, storyboards, and much more. Porous-tipped pens are available in a variety of colors with a range of tip types from ultra-fine points to brush tips to broad chisels. The ink used for porous-tipped pens is fluid, allowing for the potential to blend colors similar to watercolor painting. Forensic Document Examiners (FDEs) frequently utilize filtered light and radiation to reveal differentiating optical features of pen inks in a non-destructive and non-invasive manner. The primary drawback is the inherently subjective nature of solely relying on visual examination of the detected optical characteristics. The blending feature of porous-tipped pens can also lead to difficulties for FDEs due to the change in color as a result of blending ink in artwork. Gray value analysis and HSI are proposed as objective approaches that can help increase transparency and confidence in pen ink discrimination. There is little research regarding the use of these two methods for pen ink analysis; however, HSI has been established as a useful method among art conservationists to analyze artistic manuscripts, drawings, and paintings.

There is a need within the field not only to further investigate newer types of porous-tipped pens and their blending capabilities, but also to continue to develop more efficient methods to analyze ink samples non-destructively and objectively. It is hypothesized that the combination of gray value analysis and HSI will aid in discriminating between porous-tipped ink samples. The goal of this project is to determine the most efficient protocol to objectively characterize and differentiate blended porous-tipped pen inks based on their optical characteristics. A protocol that utilizes the high-resolution imaging system by means of a Video Spectral Comparator (VSC) is being developed consisting of a combination of the most efficient illumination types and filters to maximize the discrimination of porous-tipped marker samples for a given color family. An objective and reliable image analysis approach is established based on gray value measurements and HSI that derives numerical data from images collected with the VSC.

Porous-tipped pens from five different brands were chosen for this project, as well as four different types of paper to determine if the paper medium influences the optical characteristics of the ink samples. Ink stroke samples using six colors from each brand were used to create 120 total samples. Several blended ink samples were also created as unknowns to test the proposed methods. Visual examination was performed to determine if samples could be differentiated based on color alone. All samples were recorded using the VSC under eight filters. Illumination conditions such as Infrared (IR) absorbance, IR luminescence, and Ultraviolet (UV) fluorescence were used to discriminate between ink samples in the same color family. The gray values of samples that underwent IR reflectance and IR luminescence were measured using ImageJ software. All samples were further examined under the HSI feature of the VSC to obtain an image cube, and reflectance visible spectra were collected. It was observed that both gray value analysis and HSI separately increased the number of differentiations compared with visual examination and filtered light examination. This project is a part of a broader study to investigate, develop, and propose potential methods, both traditional and novel, to analyze porous-tipped pens.

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#### Questioned Documents, Ink Analysis, Image Analysis

## J20 Quantitative Support for Forensic Document Examination in an Open Set Using Random Forests

Madeline Q. Johnson, BA\*, Iowa State University, Ames, IA 50011; Danica Ommen, PhD, Iowa State University - Statistics, Ames, IA 50011; Alicia L. Carriquiry, PhD, Center for Statistics and Applications in Forensic Evidence - Iowa State University, Ames, IA 50011

**Learning Overview:** The goal of this presentation is to illustrate how the statistical method of random forests can be used to quantify whether a pair of handwritten documents are from the same or different source based on clustered features in handwritten documents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the work of current forensic statistics relating to comparing documents in an open set.

Handwriting analysis is conducted through the expertise of Forensic Document Examiners (FDEs) by visually comparing writing samples. Through their training and years of experience, FDEs are able to recognize critical characteristics of writing to evaluate the evidence of writership. In recent years, there have been incentives to further investigate how to quantify the similarity between two written documents to support the conclusions drawn by FDEs.

One way to extract information from these documents is to extract various features within handwritten samples. Using an automatic algorithm with the “handwriter” package in R, a sample can be split into “glyphs,” which are small units of writing.<sup>1</sup> These glyphs are sorted into 40 exemplar groups or “clusters.” The clusters have similar structures found in documents throughout a database with many writers. Previous work related to the number of glyphs per cluster focused on quantifying the probability a questioned document was written by one of the writers in a closed set. In these cases, all of the potential sources of the handwriting are assumed to be known.<sup>2</sup> This project aims to use simulated data to study how classification tools can be used to assess the within-writer versus between-writer hypotheses in an open set of documents.

Specifically, a statistical model can be used to study the proportion of these glyphs categorized within each of the 40 clusters for each document. Then, given two questioned handwritten documents, it is possible to quantify how similar the proportions across clusters are using a distance measure, such as the difference in proportions for each cluster. Since writers over time and across documents have similar writing patterns, it is expected that the proportion of glyphs classified to these clusters is comparable when written by the same person. Conversely, the proportion of glyphs by cluster will be less similar when the documents do not share the same source. A random forest is a statistical classification algorithm made up of many decision trees that is able to use these measures to classify pairs of documents as coming from the same source or different sources when trained on previous data where the true identification was known. Results of using random forest algorithms have shown clear discernment between simulated data distance measurements from the same writer and different writers.

Findings from this statistical research provide insight on another way to quantify the similarity between two questioned documents when all possible sources are unknown.

### Reference(s):

1. Handwriter Package. *Handwriter*, 2020. <https://csafe-isu.github.io/handwriter/index.html>.
2. Crawford, Amy. *Bayesian hierarchical modeling for the forensic evaluation of handwritten documents*. PhD diss., Iowa State University, 2020. <https://lib.dr.iastate.edu/etd/18078>.

### Statistics, Random Forests, Document Analysis



## J21 Methods and Materials Used in Steganographic “Invisible Ink” Communications in Prison Facilities

*Michael T. Adkins, MS\*, Federal Bureau of Investigation, Washington, DC 20535; Heidi H. Harralson, MA\*, Tucson, AZ 85728; Larry S. Miller, PhD, East Tennessee State University, Johnson City, TN 37614*

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**Learning Overview:** The goals of this presentation are to: (1) define and describe types of invisible ink steganographic messages both produced and received by prison inmates; (2) aid in establishing patterns of production commonly used by inmates; and (3) provide research information that can assist prison facilities in detecting invisible ink communications more effectively.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing strategies to improve operational effectiveness in prison facilities and forensic laboratories in detecting and deciphering invisible ink communications.

The goals of this study are to: (1) define and describe types of steganographic messages in the form of “invisible ink” both produced and received by prison inmates; (2) aid in establishing patterns of production commonly used by inmates; and (3) provide research information that can assist prison facilities in detecting steganographic “invisible ink” communications more effectively.

This study examined steganographic messages both produced by and received by inmates in prison facilities that use invisible ink formulas to hide messages in ordinary-looking, paper-based documents. Little has been formally published in research about invisible ink or steganography used in prison communications. An earlier study examined the techniques used to develop or render visible a variety of invisible ink formulas commonly used in prison facilities. The present study examined the methods and materials commonly used in creating steganographic messages involving invisible ink. The invisible ink formulas are made of biological materials easily accessible to inmates (i.e., biological and chemical fluids, vegetable and fruit juices). Techniques of developing invisible ink messages received in prison are also accessible to inmates and include heat from a cigarette lighter or iron or the use of a small ultraviolet light.

This study evaluated a collection of steganographic messages received for analysis by a forensic laboratory to establish patterns in the method and materials that inmates use to produce invisible ink messages. The steganographic communications were evaluated on three levels. The first two levels included a materials analysis: (1) ink formulas, including the chemical components used and the method in which the ink is developed or rendered visible; and (2) substrate, including paper types used in the notes and what is accessible or received by inmates. The third level in which the steganographic messages were evaluated involved the method or form of writing or code that was used to transmit the message. For example, the messages can include handwriting, hand printing, or coded writing produced with invisible ink, swabbing parts of an innocuous communication with invisible ink to produce a hidden message, or writing hidden messages or a code in invisible ink in portions of a letter, coloring book page, envelope, postcard, or artwork.

Results of the analysis aids in establishing patterns of production commonly used by inmates, information that can assist prison facilities in detecting invisible ink communications more efficiently.

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### Invisible Ink, Steganography, Code Writing



## J22 The User's Experience (UX) Process: Website Design for the Private Practitioner

*Meredith DeKalb Miller, MFS\*, Davenport, IA 52803*

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**Learning Overview:** After attending this presentation, attendees will have gained insight into developing a website/landing page using the UX process. This process synchronizes human interactions within an operational system, increasing human well-being and providing optimal system performance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how human factors interact with elements of a system focusing on the UX with purposeful design for websites, landing pages, and mobile apps.

Forensic Document Examiners (FDEs) typically work for government laboratories, as solo practitioners, or a hybrid of both. Statistics indicate that private FDEs charge among the lowest hourly rates (\$260 USD/hr) of all the expert fields but typically receive more engagements per year with an average of 34.<sup>1</sup> Finding a qualified and suitable private examiner relies on marketing, social media, expert referral services, and word of mouth.

Creating good content for a website or social media post depends on the ideal buyer for the services. What companies/industries are connecting to the website? What solutions are they looking for? Once these questions have been addressed, a more detailed picture emerges of how to engage a client and provide a positive UX with the information.

User design interface or UX puts your client's needs first by creating an intuitive process from start to finish. A client's first interaction with an expert is reviewing their website. Websites that lack cohesion, are poorly designed, share outdated information, and are difficult to navigate do not value their client's experience. If an attorney or client becomes frustrated trying to extract relevant information, the potential client may never even contact the expert!

Too often, the business side of a private forensic examiner's workload, especially marketing, eats up an examiner's time and budget, but is rarely discussed. Through this process, experts will learn how design impacts a user's intuitive interaction with information. Focusing on the UX with a website specifically, attendees will understand the philosophy and process behind developing a website from this perspective. From first iteration to final concept, key ideas concerning content, wording, design, and branding are built into the process. Considerations of costs, time, and resources will also be discussed.

Incorporating quality design while focusing on the UX should be part of every branding strategy from the solo practitioner to larger private or government forensic laboratories. A UX directly affects their perceptions positively or negatively about an agency, company, service, and brand. Managing, allocating, and enhancing organizational resources in the most optimal way creates a mainstream process, thereby creating a quality experience for clients and contributors. Attendees will be encouraged to incorporate a dynamic campaign through layout, framework, and design while focusing on the most important factor: the user.

### Reference(s):

<sup>1</sup>. 2019 Expert Witness Fee & Practices Survey Report. ExpertPages.com.

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### Marketing, Business Development, Expert Retention and Selection

## J23 The Application of the Data Augmentation Technique to the Data Generation in Handwriting Classification

*Yoko Seki, MA\*, National Research Institute of Police Science, Kashiwa-shi, Chiba 277-0882, JAPAN*

**Learning Overview:** After attending this presentation, attendees will understand the effectiveness of the comparison between handwritten letters that were different in the letter type but similar in shape in writer classification using a neural network.

**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 techniques along with deep learning have been advancing rapidly and their application to forensic document examination has also been expected. Writer classification experiments using a neural network, conducted as one of the feasibility studies, were reported in this presentation.

Handwriting classification experiments using LeNet were done to investigate the effectiveness of using handwritten samples that were different in the letter type but similar in shape as the known handwriting. Samples used for the experiments were lowercase letters “h” and “n” written by ten Japanese writers repeated five times. Image data of samples were obtained as 8-bit grayscale images under conditions where the image size was 32 pixels x 32 pixels with a resolution of 85 dots per inch (dpi).

Experiments were done as follows: 50 samples of either “h” or “n” for the test (five samples per writer x ten writers) and 1,000 samples of the other “n” or “h” generated by Data Augmentation (DA) for the training (100 samples per writer x ten writers), ten samples of either “h” or “n” for the test (one sample per writer x ten writers) and 50 samples of the other “n” or “h” for the training (five samples per writer x ten writers), ten samples of either “h” or “n” for the test and 1,000 samples of the same letter generated by DA and ten samples of either “h” or “n” for the test and 40 samples of the same letter for the training (four samples per writer x ten writers). Average F-measure was 0.4516 (max: 0.7333, min: 0.2666). Classification using “h” only showed higher F-measure value than others. Two writers showed higher recall value than others in all experiments. Their handwriting showed the unique writing manner observed both in “h” and “n.” One writer’s samples were small in size and written at the center of the writing box, which were not observed in other writers’ samples. Moreover, the letter shape was similar between “h” and “n” in the upward stroke and the stroke terminal. The other writers’ samples were thin in ink color, though all writers used the same kind of pen and ink. On the other hand, there were “h” and “n” samples that were written by the same writer and were similar in the letter shape but showed low recall value.

These results may show that the feature extraction was applied not only to the shape but also to the placement, the size, and the line quality. Thinking of the low recall value of the writers whose “h” and “n” were written similarly in shape, the local similarity in the letter shape was less emphasized than the placement, the size, and the line quality in these experiments.

Writer classification using the local similarities in the shape between two different letters was not so effective in the experiments done. More experiments such as classification using samples with higher resolution or using more complicated algorithm will be necessary for the future study.

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### Handwriting Classification, Neural Network, Data Augmentation



### **J24 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 is offered in Istanbul to undergraduate forensic science students by academicians with a professional expertise in the field of more than 20 years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by presenting the details about the one-and-only document analysis teaching and training program at an 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 our expert establishments, whether private or governmental, and our crime laboratories into organizations that are accredited and well-known in Europe and around the world, and also 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/signature identification is one of them. An elective course is offered at 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) theoretical and practical aspects of handwriting as forensic evidence; and (4) 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), American Society for Testing and Materials (ASTM) E1732, and European Network of Forensic Science Institutes (ENFSI) and learn the operation and practical use of various instrumentation used in the field such as the Video Spectral Comparator (Foster & Freeman VSC 8000), Leica® Z6 Stereo Macroscope, and Leica® FSC Comparison Microscope.

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#### **Document Analysis, Signature Identification, Forensic Science Education**

## J25 Writer's Cramp and Technology: Its Influence on Intra-Writer Handwriting Variability

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**Learning Overview:** After attending this presentation, attendees will have learned about writer's cramp, how technology is influencing handwriting and causing an increase in writer's cramp, and how writer's cramp influences handwriting when evaluating variance and dysfluency in forensic handwriting examination cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting data about conditions that adversely affect handwriting fluency and increase intra-writer handwriting variability.

Forensic handwriting examiners evaluate handwriting fluency and range of variation to make determinations about handwriting identification. The spasmodic and temporary conditions caused by writer's cramp create a seizure in the hand muscles that control handwriting coordination, thereby retarding handwriting fluency. Such conditions need to be carefully evaluated and researched in order to differentiate them from the dysfluency that is sometimes present in handwriting simulation and other conditions.

Recent research has shown that with the increased use of hand-held technology, such as cell phones, there has been a corresponding increase in musculoskeletal disorders, including writer's cramp. Studies have shown that Cumulative Trauma Disorders (CTDs) and texting tendinitis are present among those who use hand-held technology devices on a continuous basis. While writer's cramp can be a problem for writers of all ages, historically it has tended to have an onset in adults around the age of 40. Research data is relatively limited about the prevalence of writer's cramp among younger adults.

The aims of this study are to show the connection between use of technology and the increase in writer's cramp among college students and how the writer's cramp deteriorates and increases intra-writer handwriting variability. In the study, 54 college students (mean age 21.6; median age 20) answered a questionnaire about personal technology use and whether their hands cramped. Each student wrote a pre-test sentence in handwriting and hand printing and a signature before completing a test in a criminal law class that required continuous handwritten responses. The time each student spent on the test was recorded, which ranged between 40–86 minutes. Average test writing time was 71 minutes. After the test, the students completed a second questionnaire that included handwriting and hand printing a post-test sentence and signature and answering a question about whether their hand cramped during the test.

The handwriting samples were evaluated for changes in handwriting before and after the test and were categorized into three groups: severe, moderate, and slight/no change in handwriting. Evaluation of the samples showed that 42 of the 54 students (77%) exhibited moderate to severe deterioration in handwriting between pre- and post-test. Changes in the handwriting between pre- and post-test samples included tremor, size, alignment, slant, proportion, and other factors. Among the 42 students, 39 stated they experienced writer's cramp during the test. Participants in the survey complained of pain, fingers "locking up," spasming, and burning sensations during the test. Students were observed massaging their hands during the test and some students stated that their hands were not working. On average, the 42 students showing moderate to severe handwriting deterioration reported that they spent over four hours a day using their cell phones.

Results of this study show that there is evidence of early onset of writer's cramp among college students. Results support prior research showing that the decline in using and teaching handwriting coupled with the increased use in hand-held technology has caused a deterioration in handwriting and a corresponding increase in writer's cramp and intra-writer handwriting variability.

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### Forensic Handwriting Examination, Handwriting Variation, Writer's Cramp



## **J26 History and Recent Revisions to the Policies Regarding Testimony of Forensic Document Examiners in the State of Texas**

*Keelie Johnson, BS\*, Austin, TX ; Sarah Pryor, Texas Department of Public Safety Crime Laboratory, Austin, TX 78752; Angela Roe, MSc, Texas Department of Public Safety, Austin, TX 78752*

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**Learning Overview:** After attending this presentation, attendees will have an understanding of the policies and procedures set forth by the Texas Legislature throughout the years and the impact on forensic document examiners stating their expert opinions for the courts in Texas.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information to attendees about the Texas Forensic Science Commission (TFSC), the past and recent updates to laws governing testimony of experts in the state, and the effect of these policies on forensic document examiners when testifying in Texas.

In 2005, the TFSC was established during the 79<sup>th</sup> Texas Legislative Session. It is the TFSC's responsibility to investigate professional negligence and misconduct that may impact the quality of results for forensic analyses in accredited laboratories, facilities, or entities. Passed in May 2005, House Bill 1068 (HB-1068) set forth that Chapter 38 of the Code of Criminal Procedure will be amended. The addition of Article 38.01 discusses in detail the creation, composition, and further duties of the Commission.

HB-1068 also details the amendments to Article 38.35, Code of Criminal Procedure: Forensic Analysis of Evidence; Admissibility. The accreditation of laboratories and the law surrounding the admissibility of evidence fell under the Government Code 411.0205. The Texas Legislature stated that forensic analysis of physical evidence and expert testimony relating to the evidence would not be admissible in criminal court unless the crime laboratory was accredited at the time of analysis.

Later in 2013, Senate Bill 1238 (SB-1238) was passed by the Texas Legislature. It provided updates to the responsibility of the Commission. Now, the Commission is able to investigate claims of professional negligence and misconduct involving forensic disciplines that are exempt from accreditation.

With the implementation of Senate Bill 1287 (SB-1287) in June 2015 at the 84<sup>th</sup> Texas Legislative Session, the responsibility of accrediting crime laboratories was transferred from the Texas Department of Public Safety to the TFSC. This became effective September 1, 2015. The bill also requires that all practicing forensic analysts be licensed in the State of Texas starting January 1, 2019. Some forensic disciplines are exempt from this licensing requirement if they are also exempt from the Commission accreditation requirement by administrative rule.

Throughout the years, amendments to Texas Administrative Code, Rule 651.7 would exclude particular forensic disciplines from the accreditation requirement by the administrative rule. In January 2019, the TFSC met to discuss the constitutionality of Forensic Document Examination being included in the accreditation policy. The only three examiners in the State of Texas able to meet the legal requirements set forth by HB-1068 were in the Texas Department of Public Safety Crime Laboratory system. Defense attorneys in particular were taking issue with the fact that they could not hire their own expert witnesses. When the TFSC reconvened in May 2019, it was decided that it was not constitutional to uphold HB-1068 and Forensic Document Examination was removed from the accreditation standards, and therefore, the licensing requirements. The amendment was made to the Texas Administrative Code in July 2019.

This presentation will go into detail about the policies and requirements surrounding accreditation and licensing. It will be discussed how these procedures impact Forensic Document Examination testimony in the courts for the State of Texas. Also, this presentation will further explain the TFSC and how they function as the presiding authority over forensic analysts.

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### **Forensic Document Examination, Testimony, Laws**



## J27 Relationships Between Handwriting Slant and Demographic Features

*Anyesha Ray\**, Center for Statistics and Applications in Forensic Evidence, Ames, IA 50011; *Alicia L. Carriquiry, PhD*, Center for Statistics and Applications in Forensic Evidence - Iowa State University, Ames, IA 50011; *Danica Ommen, PhD*, Iowa State University - Statistics, Ames, IA 50011

**Learning Overview:** The goal of this presentation is to use statistical analysis to explore where there is a relationship between demographic features, such as handedness, gender, and age, and attributes of a person's writing, such as slant.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing statistical support for relationships between demographic information and handwriting characteristics, such as slant.

While studies have been conducted on whether there are relationships between handwriting and demographic features, this study aims to provide statistical support for the relationships to handwriting slant specifically. Handwriting samples were collected from 90 adults.<sup>1</sup> Each participant completed three data collection sessions, each at least three weeks apart. Participants were asked to write three different prompts that have three different lengths: the London Letter being the longest, followed by an excerpt chosen from the book *The Wonderful Wizard of Oz*, and the phrase "The early bird may get the worm, but the second mouse gets the cheese" being the shortest. At each session, a survey was completed, and three writing prompts were each transcribed three times resulting in nine pages of samples each session. In total, there are 2,430 handwriting sample images as well as demographic-specific information collected from surveys for all 90 participants. Survey data include information about the participant's handedness, age group, gender, location of third-grade education, and time of day in which the writing sample was taken.

These handwritten images are then broken into smaller segments of writing that are called "graphs" or "glyphs" by the handwriter R package.<sup>2,3</sup> Information such as curvature of the glyph, lengths, and heights, as well as shapes of the loops was extracted. Following the methods described in Crawford, these glyphs are then grouped according to their basic shape into 40 different clusters.<sup>4</sup> For each glyph in a cluster, a quantitative measure of slant is computed. The slant of a writer is determined by calculating the direction of greatest variability in a letter using principal component decomposition and the angle of rotation corresponding to that direction. This is called the rotation angle of a glyph, which is a numerical value between 0 and  $2\pi$ . In this study, the rotation angle of all the glyphs within a cluster for each handwriting sample was averaged as summary information. The average rotation for all glyphs across all the handwriting samples for each person was also calculated. Then, a regression was fit with the demographic information as explanatory variables and the average rotation angles as the response. Because rotation angles live on a circle, the regression model is based on a probability model appropriate for this type of data.

Based on exploratory analysis, it is hypothesized that females will have more of a slant in handwriting than males, left-handed writers more of a slant than right-handed, and younger age groups will have more of a slant than older age groups. In summary, this presentation aims to use statistical analysis to determine if there is a relationship between demographic features and slant.

### Reference(s):

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### Handwriting, Statistics, Handwriting Features

## J28 Remedial Paper Examinations

Larry A. Olson, MFS\*, Internal Revenue Service National Forensic Lab, Chicago, IL 60607

**Learning Overview:** The goal of this presentation is to remind document examiners of some of the recommended basic techniques of paper examinations, to expose them to others they may not be aware of, and to provide information on the frequency of use of methods within the discipline.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing the competence of document examiners through familiarity with techniques regarding paper examinations.

All routine document examinations begin with paper. Several standard references list various methods for examining paper.<sup>1-3</sup>

The Scientific Working Group for Forensic Document Examination (SWGDOC) Standard for Non-Destructive Examination of Paper guidelines lists 20 discrete/different examinations, paraphrased as follows: (1) 7.5—using transmitted light for watermarks; (2) 7.6—for color; (3) 7.7—for thickness; (4) 7.8—for Ultraviolet (UV) fluorescence and Infrared Luminescence (IRL); (5) 7.9—for chemical or other contamination, alterations, and carbonless paper transfers; (6) 7.10—for relative opacity; (7) 7.11—for surface texture; (8) 7.12—for length and width; (9) 7.13—for weight; (10) 7.14—of corners and evaluate angles (for example, squared, curved, rough finish); (11) 7.15—of edges (for remnants of binding, adhesives, or padding material); (12) 7.16—of edges for manufacturing markings (for example, cut marks, striations or coloration); (13) 7.17—of lines or other printed material (for line length, spacing, and for broken or deformed patterns); (14) 7.18—for security features (for planchettes or security fibers); (15) 7.19—for carbonless paper chemicals and evidence of a carbonless system; (16) 7.20—for trace materials (for example, opaqueing solution, correction strips, tape, or other materials); (17) 7.21—for surface damage due to abrasions, handling, storage, or other physical changes (for folds, creases, crimp markings, fiber disturbances, or other relevant characteristics); (18) 7.22—for staples and staple holes; (19) 7.23—for perforations, hole punches, or other torn portions; and (20) 7.24—for indentations (of handwriting, clipboard marks, paper clip impressions, and other extraneous markings).<sup>4</sup> A demonstration of the paper making process will show how certain characteristics occur.

**Routine Examinations:** It is assumed three of the above examinations are routinely done by all examiners: (1) examinations for watermarks (to determine the manufacturer, and possibly the date of the paper's manufacture); (2) examinations with UV and IR illumination (to determine the fluorescence/luminescence characteristics to evaluate a common source); and (3) examinations for indentations of handwriting, paper fiber disturbances, etc.

The determination of the manufacturer by watermark requires some research, but can be fruitful, as it may allow determination of a common source or the date the paper was made. This presentation will summarize a couple of different types of watermarks and suggest a streamlined method and suggest useful contacts to facilitate this research.

Hilton long ago cautioned that differences in fluorescence between questioned sheets of paper do not necessarily indicate a different source, because of the variations inherent in the manufacturing process.<sup>5</sup> This presentation will illustrate the manufacturing and conversion process and the reasons for difference in fluorescence.

Indentations may be caused on paper from a variety of sources, especially handwriting. Some indentations may be created on the blank paper stock during the paper conversion process. This presentation will present examples to look out for.

**Less Routine Examinations:** Several of the examinations are possibly less routinely done, such as measuring papers for thickness (7.7), opacity (7.10), and weight (7.13). These require more specialized instruments, which may need to be calibrated, and many labs nowadays have procedures involving calibration. Measuring the size of a document for its length and width (7.12) is relatively easy and may help determine its geographical origin (that is, North American or European). But, unless the paper is of an untypical or unfamiliar size, is it routinely performed?

In addition to measuring the dimensions, a third group of examinations, if performed, may indicate whether a paper has been trimmed or modified in some way (1) examining the document corners and evaluate angles (7.14), and (2) microscopically examining edges for remnants of binding, adhesives, or padding material (7.15) or for cut marks or striations or coloration (7.16).

In the absence of obvious evidence of alteration, are these examinations done by many?

This presentation will conclude with the results of a survey done within the discipline on the prevalence of these methods above and the tools used.

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5. Hilton, O. Pitfalls in the Use of Ultraviolet Examination to Differentiate Between Writing Papers. *J Criminal Law and Criminology* 1949:40(4):519-522.

## Paper Examinations, Watermarks, Machine Indentations

### J29 Digital Walkthrough of the Validation of a Method for Measuring Magnetic Flux of Toner-Printed Documents

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**Learning Overview:** After attending this presentation, attendees will better understand the potential for the 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, including how to set up the instrument, implement a relevant test method, troubleshoot and optimize their laboratory protocol, and assess the performance of the method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method for quickly examining black-and-white toner-printed documents, reducing the need for more time-consuming or destructive methodologies.

This study presents the culmination of a body of work on the magnetic properties of toner. In the first phase of research, the stability of magnetic flux fields over time were assessed, as well as measurement repeatability and the amount of variation present in a representative toner population sample. In the second phase of research, the instrument and method parameters that affect measurement precision and accuracy were assessed. In the third phase of research, measurement signal suppression and enhancement caused by hysteresis effects and other biasing induction current spatial effects were investigated. In the fourth phase of research, measurement repeatability and reproducibility were explored via an inter-laboratory study with a defined methodology optimized based on the parameters found to be optimal in phases one through three. The fifth and final phase of research was conducted with those studies as the foundational basis, and in this phase of research the refined analytical method developed and employed in the fourth phase of this study was independently validated. During the fifth phase of research, a known reference material was developed for use with the magnetic flux measuring device to assist in assessing the precision and accuracy of measurements. This material was synthesized using a three-step process that involved resin synthesis, magnetite synthesis, and toner aggregation. The analytical method was then validated on a representative population sample consisting of 54 toner-printed document samples collected from different devices, using the known reference material as a positive reference material in addition to the previously utilized positive and negative quality control samples. During the validation study, the bias and precision, limit of detection, limit of quantitation, suppression and enhancement, and stability of measurements were assessed.

It was found that magnetic flux measurements of toners were stable over time, measurements were repeatable, and the variation between different toner samples within the population collected for this study was large enough to appreciate the relative rarity of the measured magnetic flux value, though there were some instrument and method parameters identified that could cause inaccurate or imprecise measurements. These parameters could be controlled by the implementation of a standard analytical methodology. Magnetic flux measurements of toner-printed documents conducted via a standardized analytical methodology in conjunction with a known reference sample were found to provide a promising method for deployment in forensic laboratories. The method proposed is rapid, non-destructive, requires no sample preparation, provides numeric results that can be objectively interpreted, and does not require costly consumables.

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## Questioned Documents, Toner, Magnetic Flux

## J30 The European Document Experts Working Group (EDEWG) Organization and Service

*Andreas J. Rippert\**, St. Gallen, SG, SWITZERLAND; *Jürgen Bügler, PhD\**, Bavarian State Bureau of Investigation, Munich 80636, GERMANY; *Rolf Fauser\**, State Criminal Office Baden-Wuerttemberg, Stuttgart 72372, GERMANY; *Hakon Schjonsby\**, National Criminal Investigation Service, Oslo N-0034, NORWAY; *Kairi Kriiska\**, Estonian Forensic Science Institute, Tallinn 13419, ESTONIA

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**Learning Overview:** After attending this presentation, attendees will have learned how the EDEWG is organized and how others can interact with their knowledge, how information is spread to all the members, subgroups with their topics, setup of collaborative exercises, and practical training in workshops.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the EDEWG supports the aims and objectives of the European Network of Forensic Science Institutes (ENFSI) in the area of forensic document examination.

The activities of EDEWG will be directed toward: exchange of information and expertise, support on technical issues, promoting quality assurance, proficiency testing and collaborative exercises, harmonization of examination procedures and techniques, and cooperation in research and development

EDEWG is managed by the Steering Committee (eight experts from different institutes and different European countries). The chairman as a member and head of the steering committee has a four-year term of office. The working group consists of 61 ENFSI members from 32 different countries and 11 associate members. A conference is held every two years and business meetings are held annually, in conjunction (where possible) with the EDEWG Conference. During the annual business meeting, besides other administrative duties, the voting of the steering committee takes place. The duration of the conference is normally 3.5 days comprising one day of workshop, oral presentations, closed sessions, poster sessions, and hands-on as well as presentations of new equipment during the meeting as a road show by suppliers. The information of all our members is organized through our own digital teamsite, including a communication platform, where one can message expert to expert.

EDEWG has a number of mainly technical-based subgroups, each taking forward areas such as research and development, method validation, writing of methods and procedures, quality assurance-trials, exchange of information, collection of samples and information for database purposes, etc., in specified areas of questioned documents. At the moment, the topics of these subgroups are: (1) non-destructive examination of printing products (2) analysis of inks and toners; (3) document dating; and (4) security documents recognition.

Each year, EDEWG produces one quality assurance trial to distribute among its members. The evaluation of the results helps the Steering Committee to decide if, for example, workshops are necessary to train its members or if existing best practice methods need to be adapted or new methods written.

This presentation will deal with organizational structure of EDEWG inside of ENFSI. Attendees will learn about the Steering Committee and their obligations. The annual tasks of EDEWG, such as Conference, business meeting, collaborative exercises, and renewal of best practice methods will be presented. Furthermore, all of the subgroups will point out one of their hot topics at the moment and how they organize further educational trainings. Attendees will obtain a view on the EDEWG teamsite, which is also being used as a communication platform.

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### EDEWG, Document Experts, Communication Channels



## J31 Generating and Harnessing Objective Data for Trash Mark Examinations

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**Learning Overview:** The goal of this presentation is to demonstrate the foundational validity of trash mark comparison methods through empirical, objective data. By harnessing this objective data, forensic scientists can define and apply mathematical calculations and statistical approaches to make the analysis more transparent and reproducible.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining a method to move trash mark examinations toward pre-defined decision criteria and data analyses. This presentation will discuss an on-going effort to digitize documents and accurately record trash mark data using a reproducible method. This method also provides an improvement in efficiency, especially when numerous known machines with multiple functions are being compared to one or more questioned documents.

Forensic scientists periodically face challenges regarding the lack of foundational research and the subjectivity of their methods. Pattern recognition methodologies, in particular, require application of significant training and experience to make subjective decisions. One such pattern recognition method is the use of printed or photocopied defect marks, commonly referred to as trash marks, from office machines to associate a questioned document to a source. This method may assess whether documents share a common source (document to document comparison) or whether a document may have been produced using a specific machine (document to machine comparison). This type of examination has been in use in forensic laboratories for decades, yet it remains qualitative and subjective in nature.

An evaluation of different approaches to digitization (e.g., scanning versus photography) of questioned documents and exemplars will be presented, along with the variables to consider for each approach, the pros and cons of each, and a recommended procedure to follow based on how the images will be used. Next, the development of a custom software that serves as an objective, reproducible tool for examiners to utilize as part of trash mark examinations will be presented. This software includes core functionality such as finding the trash marks in a digital image and recording the location and size as well as capturing an image of each trash mark. This enables comparison of morphology across multiple exemplars and with questioned documents. In addition, the software performs comparison of trash mark constellations between two or more pages. A demonstration of the software will be provided, followed by a discussion to gather feedback for important parts of the software design.

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### Trash Marks, Macro Development, Foundational Research

## J32 The Effects of Heating and Laser Printing on Paper Surface Textures as Assessed by Discrete Two-Dimensional Fast Fourier Transforms and One-Dimensional Power Spectra

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**Learning Overview:** After attending this presentation, attendees will understand the value of the discrete two-dimensional fast Fourier transform in documenting the surface textures of paper samples, such as common office and copier papers. Attendees will also understand how the two-dimensional fast Fourier transforms (2D-FFT) can be converted into one-dimensional power spectra (1D-PS), which are convenient for comparing different paper samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that paper surface textures are not adversely affected by temperature and passage through laser printers.

The manufacture of modern paper products from pulp typically involves a series of steps: extrusion of the pulp onto a wire screen, removal of water (dewatering), vacuuming with a suction roll, and drying while being pressed with canvas fabric against a drying roll.<sup>1</sup> These forming and drying processes impart periodic patterns of marks to the finished sheet of paper. Because different paper manufacturers use different machinery, it is possible to differentiate their products from one another using 2D-FFTs.<sup>1-3</sup>

Previous research on paper textures has relied on scanning the textures using transmitted light.<sup>1-3</sup> However, not all flat-bed scanners have light sources with sufficient intensity to acquire usable transmitted light images through single sheets of paper. This is particularly true of the widely used all-in-one printers, which also incorporate scanners. One objective of this research was to determine if reflected light images of sheets of paper could provide 2D-FFTs capable of distinguishing different brands of office and copier paper.

Ten brands of office and copier paper were used in this research project. Paper surface textures were scanned in reflectance mode as bitmap images at a resolution of 600 dots per inch (dpi). Two scanners were used: an Epson® Perfection V500 Photo flat-bed scanner and an Epson® WorkForce WF-3640 all-in-one printer. The Epson® scanner and Epson® all-in-one printer were controlled by Epson® Scan software (version 3.7.7.0). ImageJ software version v.1.4.3 was used to equalize and normalize the histograms of the scanned images of paper surface textures. An ImageJ macro modified from Sasaoka et al was used to compute all 2D-FFTs and 1D-PS.<sup>3</sup> The paper brands examined in this project could be readily distinguished by their 2D-FFTs and 1D-PSs obtained by scanning in reflectance mode.

To assess the effect heat on the surface textures of paper, paper samples were heated in the oven of a conventional domestic gas range. Two temperatures were selected for this part of the study: 149°C (300°F) (the temperature at which paper begins to turn brown) and 216°C (420°F) (a temperature just below the autoignition temperature of most papers).<sup>4</sup> Samples were held at each temperature for one hour. The oven temperatures were monitored using a Digi-Sense® Digital Thermometer with a Type K thermocouple. The calibration of the digital thermometer was National Institute of Standards and Technology (NIST) certified. Six 4.0cm X 4.0cm areas were scanned on each paper sample. These areas were located at the upper left, upper right, center left, center right, lower left, and lower right on each page. The 2D-FFT and 1D-PS were calculated for each sample using the six scans. The paper samples heated to 149°C (300°F) showed only slight discoloration, while those heated to 216°C (420°F) were friable and a yellow or brown color. It was found that the 2D-FFT and the 1D-PS were not altered by heating.

In laser printing/copying, toner particles are transferred to the paper surface, then fused to the surface with an elevated temperature. A Canon® imageRUNNER ADVANCE C5560 printer was used to copy a template with black borders, a vertical black stripe, and small colored blocks (red, blue, and green) onto samples of each of the brands of office and copier paper. The surface texture of each sample was scanned before and after laser copying. The surface textures in the areas where no toner was deposited were unaffected. The laser copier embossed a grid pattern on the fused toner particles. This grid pattern contributed to the 2D-FFTs and 1D-PSs of the paper surfaces but did not obscure the contributions of the paper surfaces.

This project demonstrated that 2D-FFTs and 1D-PSs capable of differentiating brands of office and copier paper can be obtained from paper surfaces scanned in reflectance modes in flat-bed scanners and popular all-in-one printers. The 2D-FFTs and 1D-PSs are not affected by heating paper samples close to their autoignition temperature. The 2D-FFTs and 1D-PSs are not affected by passage of the paper through a laser printer.

### Reference(s):

1. Myata H., Shinozaki M., Nakayama T., Enomae T. A discrimination method for paper by Fourier transform and cross correlation. *J Forensic Sci* 2002;47:1-8.
2. Berger C.E.H., Ramos D. Objective paper structure comparison: Assessing comparison algorithms. *Forensic Sci Int* 2012;222:360-367.
3. Sasaoka S., Saito K., Higashi K., Limwikrant W., Moribe K., Suzuki S., Yamamoto K. Design of one-dimensional power spectrum using two-dimensional fast Fourier for discrimination of paper-based kraft tapes. *Forensic Sci Int* 2015;257:329-336.
4. Café, T. *Physical constants for investigators*. [www.tforensic.com.au/docs/article10.html](http://www.tforensic.com.au/docs/article10.html) accessed June 24, 2020.

### Fourier Transforms, Paper Textures, Flatbed Scanners

## J33 Minimally Destructive Ink Analysis Using Miniaturized Ultraviolet/Visible (UV/Vis) Spectroscopy

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**Learning Overview:** After attending this presentation, attendees will understand a UV/Vis spectrometric methodology for the minimally destructive chemical analysis of different ink types.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method of ink classification by miniaturized UV/Vis spectroscopy that utilizes a new sampling technique in order to allow for smaller sample sizes that thereby maintain most of the physical characteristics of handwriting after chemical analysis.

Questioned document analysis often deals with the validation of legal documents and other such forms that may contain a particularly small sample of handwriting. It is possible that a Forensic Document Examiner (FDE) is faced with a sample as small as a set of initials. Conventional destructive chemical analysis is unfavorable as it often utilizes an organic solvent extraction of the entire sample. When working with a particularly small sample or a sample where it is also important to be able to do non-destructive visual and microscopic analysis of physical characteristics, an FDE may elect not to perform conventional chemical testing.

Conventional UV/Vis spectroscopy is another established method for chemical ink analysis, but faces the same issues of large sample size and original document destruction. Additionally, the conventional solvent used in UV/Vis ink analysis, pyridine, produces a nauseating odor and is a systemic toxicant.<sup>1,2</sup> UV/Vis spectroscopy is ideal in that it is possible to determine important information from a visual examination of the spectra that does not necessarily require in-depth molecular analysis.

This new analytical method utilizes a 0.7mm steel mechanical pencil tip as a micro-punch to transfer tiny ink-on-paper samples from written characters and test them on a significantly smaller scale with a micro-volume UV/Vis spectrometer. Using a safe and mild detergent as a solvent, six micro-punches generate recognizable and repeatable spectra that allow for differentiation between types of pens. Testing began with a variety of brands of black and blue ballpoint, gel, and porous point pens. The analysis later expanded to thermochroic, or “erasable,” gel pens and alcohol-based porous point permanent markers due to their forensic relevance. These samples are not commonly tested early in the development of a method for chemical analysis, but it makes sense to include them because questioned documents frequently looks at evidence material where handwriting is meant to have intense staying power, as in “permanent” inks, or is meant to be easily destroyed, as lay-people feel they achieve with pigment that deactivates. Additionally, these formulations are popular among consumers.<sup>3-4</sup>

After the micro-punch samples are taken, it is still possible to see general physical and microscopic characteristics of the characters, and samples can be taken with strategic placement in order to preserve the most relevant or distinctive aspect of any given character being examined. The larger a sample is the more spread out the six punches can be and the less it disturbs the original writing pattern; however, this method works on a sample as small as a set of initials and leaves them readable with some lines of ink still present for further analysis

The results of these tests were found to be replicable when analysis was in keeping with the set procedure. It was found that ballpoint, gel, and porous point pen inks were differentiable from one another, and the spectra for ink formulations were clearly different from the spectra obtained by running paper blanks, showing the ink spectra were notable due to the ink itself and not any paper interference. The method was additionally found to work on deactivated thermochroic pigment and on alcohol-based porous point pen inks, indicating this form of analysis is suitable for a wide variety of samples, in contrast to conventional methods. The intention of this research is to develop an accessible and replicable methodology for minimally destructive chemical ink analysis, and to use the existing testing that has been performed and further testing to establish a searchable database of reference UV/Vis spectra.

### Reference(s):

1. Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography. *ASTM International*, E 2227 – 02: 2-5.
2. U.S. EPA. *Health And Environmental Effects Profile for Pyridine*. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/X-86/168 (NTIS PB89123384), 1986.
3. *The Science Behind FriXion Erasable Pens*. Na., accessed October 4, 2020, <https://www.nippon.com/en/features/c00520/>.
4. *Sharpie—About Us*. Na, accessed October 4, 2020, <https://www.sharpen.com/about>.

### Ink Analysis, Miniaturized UV/Vis, Minimally Destructive

## J34 Force Plate and High-Speed Video Analysis of the Stamping Device Application

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**Learning Overview:** The goal of this presentation is to show the forensic science community and, in particular, forensic document examiners the utility of applied force plates and high-speed video analysis of the biomechanical action of the application of stamping devices to paper.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the potential correlation between the kinetics of stamping and the resultant stamp impression.

Stamping devices are used extensively in business, government, and personal affairs. They may be used for a variety of applications such as: addresses, business procedures, official uses, packaging, decorative applications, and others. The examination of stamps and stamp impressions are part of the work of forensic document examiners. While there have been several references that address how stamp impressions may appear from the physical act of stamping, there has not been published research into the applied forces and appearance of the stamp impressions.

The act of applying stamping devices does result in variation in the appearance of the impressions. The range of variation in stamp impressions tends to be greater than that from other mechanical or electromechanical means such as that seen from office printers. Among a number of factors, the variation is due in part to the biomechanics of manually applying the stamping device to make an impression.<sup>1</sup>

In this study, several stamping devices were used to make impressions onto a variety of substrates, including office papers, receipts, envelopes, and cardstock. A pair of stamping devices with the same stamp dies was made; one was a self-inking stamp type, the other a traditional rubber stamp requiring a separate stamp ink pad. Other common stock stamps of varying sizes that are produced in large quantities were also tested. The stock stamps were of the self-inking and traditional stamp types.

Impressions from the different stamping devices were made with the various substrates on top of Advanced Mechanical Technology Inc. (AMTI®) portable force plates with data collection done by NetForce software. Force data in the  $x, y, z$  planes ( $F_x, F_y, F_z$ ) were collected as well as rotational moment data about the  $x, y, z$  axes ( $M_x, M_y, M_z$ ) and stamp Center of Pressure (CoP) variability. During the stamping process, high-speed video was recorded using an MS 130K high-speed camera.

Examination of the stamp impressions and the data set from the force plates with high-speed video images was conducted to determine: (1) the typical range of forces applied during the stamping process; (2) whether artifacts in the stamp impressions could be correlated to the applied forces, (for example, if impressions are intentionally made with more force to one side of the stamp); (3) the range of applied force needed to make an idealized impression from a given stamp; and (4) the appearance of impressions at the extremes of minimum and maximum applied forces.

The examination of the impressions was conducted by both qualitative and quantitative means. Qualitative examinations were performed at a macroscopic and microscopic level for the impression appearance, including the presence of stamping artifacts. Quantitative analysis was accomplished by image analysis via the software ImageJ.

### Reference(s):

- <sup>1</sup> Jan Seaman Kelly. Forensic examination of rubber stamps: A practical guide. Charles C Thomas Publishers, 2002.

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### Stamping Devices, Force Plate, Forensic Stamp Impressions



## J35 A Study Into Additive Manufacturing to Clone Stamping Device Impressions

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**Learning Overview:** After attending this presentation, attendees and, in particular, forensic document examiners will be introduced to the potential of additive manufacturing, which includes in colloquial terms 3D printers, to produce a stamp that mimics a stamping device impression.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the features that may be present when a stamping device impression has been cloned via an additive manufacturing (3D printing) process. Limitations on what can be cloned via this process will be outlined.

Inked stamp impressions arise in many document-related transactions and may be subject to forensic examination. The stamp dies used to make inked impressions may be made of rubber, photopolymer, gel, or metal and are hand-applied to a variety of items such as: business documents, envelopes for postal use, invoices, receipts, legal documents, passports, and other official documents.<sup>1</sup>

While there are a variety of means that may be employed to clone a stamping device impression, there have not been published works in the forensic community on the potential vulnerabilities that stamp impressions may have to cloning attempts by additive manufacturing.

Additive manufacturing is a means to produce objects by the addition of materials instead of a traditional means whereby materials are removed by processes such as milling or other mechanical means. The lower cost and increased availability of additive manufacturing devices in the past decade may mean that additive manufacturing methods are now exploitable for the purpose of stamp impression cloning. To address this growing concern, this study investigated whether it is possible to successfully clone stamping device impressions using additive manufacturing, and whether it is possible to identify if a stamped impression on paper was simulated from a manufactured clone.

For this study, a number of stamp impressions made from new stamps and those that have been heavily used were cloned on a variety of different additive manufacturing devices. The new stamps included both custom-designed stamps with a variety of typefaces and stock stamps that are produced in bulk by stamp manufacturers. The stamps that were heavily used were decommissioned stamps obtained from a government surplus sale. Self-inking and traditional stamps, the latter requiring a separate ink stamp pad, were used in both the new and heavily used stamp types.

The impressions to be cloned were made onto a variety of papers with different degrees of printing, writing, and other interfering elements. Multiple impressions of the same stamp were made to portray some of the variations that may be expected from the stamping process. Working from the impressions only, images and subsequent rendering of files suitable to be used for the additive manufacturing process were made. Materials were selected that allowed for ease and economics of manufacture along with sufficient inking capabilities to permit stamping action onto the page.

The resultant cloned stamps were then impressed onto different papers using an ink stamp pad. Cloned impressions were then examined for their characteristics to see: (1) if any artifacts were present; (2) if such artifacts could be attributed to the additive manufacturing process; and (3) the degree of congruence to the original impression.

### Reference(s):

- <sup>1</sup>. ANSI/ASB Standard 117, Standard for Examination of Stamping Devices and Stamp Impressions, First Edition 2020

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### Stamping Devices, Additive Manufacturing, Stamp Clone

## **K1 The Effect of COVID-19 and Texas’s Phased Reopening Plan on Ethanol Concentrations in Driving While Intoxicated (DWI) Cases in Harris County, Texas**

*Grayce Behnke, MS\*, Houston, TX 77096; Teresa R. Gray, PhD, Houston, TX 77054*

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**Learning Overview:** After attending this presentation, attendees will understand the impact that COVID-19 and Texas’s phased reopening plan had on ethanol concentrations in DWI cases in Harris County, TX.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight on the effect that Texas’s phased reopening plan had on ethanol concentrations in DWI cases as Texas was one of the first states to begin reopening following a statewide shutdown due to COVID-19.

**Objective:** To compare the results from alcohol-only DWI cases from the same time frames in 2018, 2019, and 2020 to show the effects of COVID-19 and the county’s shutdown and phased re-opening plan on ethanol concentrations in DWI cases in Harris County.

**Background:** On February 11, 2020, the World Health Organization named the novel coronavirus that was first identified in Wuhan, China, COVID-19. On March 6, 2020, Harris County, TX, had its first two confirmed COVID-19 positive cases. To help stop the spread of the virus, Harris County’s chief executive, Judge Lina Hidalgo, issued a “Stay Home, Work Safe” order on March 24, 2020, which was followed by an executive order from Texas Governor Greg Abbott on March 31, closing non-essential businesses, including bars, and encouraging drive-thru, pickup, and delivery from restaurants. On April 27, Gov. Abbott began the phased re-opening of Texas, becoming one of the first states to re-open. On June 26, due to an increase in COVID-19 cases, Gov. Abbott again limited certain businesses, such as restaurants, to operating at 50% of the total listed occupancy of the establishment and prohibited businesses selling mostly alcohol beverages, such as bars and breweries, from opening.

**Method:** Alcohol-positive DWI cases from the same time frames in 2018, 2019, and 2020 were compared in order to determine the effects of COVID-19, and the county’s shutdown and phased re-opening plan on concentrations of ethanol present, number of cases, and age of suspects.

**Results:** There was a significant decrease in cases submitted to the Harris County Institute of Forensic Sciences for DWI offenses during the “Stay Home, Work Safe” order. The Harris County Institute of Forensic Sciences received approximately 35% fewer cases during this time frame in 2020 when compared to 2018 and 2019. Even though the number of cases decreased, the average ethanol concentration remained consistent at  $0.169 \pm 0.059\text{g}/100\text{mL}$ . As the county began phased re-opening at the end of April 2020, submissions rapidly returned to normal.

**Conclusion:** While the “Stay Home, Work Safe” order and state executive order limiting the state of Texas to only essential functions was in place, the overall caseload for DWI cases decreased. However, the concentrations of ethanol remained consistent with results from 2018 and 2019 during the same time period, indicating COVID-19 and Texas’s phased reopening had no effect on ethanol concentrations.

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### **DWI, COVID-19, Ethanol**

## K2 Lethal Self-Poisoning by Ingestion of Yew (*Taxus Baccata*, Taxacées): Two Case Reports

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**Learning Overview:** The goal of this presentation is to examine the interest of research toxins of yew in cases of unexplained deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of unusual poisonings with toxic plant intoxications.

**Introduction:** Yew trees, *Taxus baccata*, are evergreen trees that are widespread in Europe. Female yew trees produce black seeds surrounded by red fruits, called arils. Yew, used in certain chemotherapies, is potentially lethal in cases of ingestion. Except for the arils, all parts of yew are toxic. Presented here are two cases of yew self-poisoning.

**Clinical Cases:** The first case is a 28 year-old man who was found dead at home. He had tried to poison himself with yew 15 days before. Only round millimetric particles, appearing to be seeds, were found in the stomach during the autopsy.

The second case is an 18-year-old woman with no medical, surgical, or psychiatric history who had been admitted to intensive care after vomiting and cardio-respiratory arrest. The patient's health quickly deteriorated, and she was declared brain dead a few hours later. Fragments of green leaves were found at her home and identified by botanical analysis as yew. Similar elements were found in the stomach during the autopsy.

**Discussion:** Yew's toxicity is linked to the presence of taxines, found in varying concentrations throughout the year, with a peak in winter. It is mediated by two alkaloids, namely the taxines A, B, and their derivatives, but also by a phenolic component, 3,5-Dimethoxyphenol (3,5-DMP). Taxines, especially taxine B, have similar properties to anti-arrhythmics. The lethal dose is between 0.6 and 1.3g of leaves (1g containing 5mg of taxines) per kilogram of body weight. Symptoms appear quickly after ingestion, generally within two to five hours, and include digestive symptoms, arrhythmias, respiratory and neuromuscular troubles, all of which can lead to death.

Two techniques can be used to identify yew components: Gas Chromatography coupled with Mass Spectrometry (GC/MS) and High-Performance Liquid Chromatography/High-Resolution Mass Spectrometry (HPLC/HRMS). In case of yew poisoning, 3,5-DMP is usually found in blood and urine by GC/MS. In both cases presented, this test was negative, thus suggesting that the use of GC/MS alone may not be sufficient to detect yew intoxication. Yew-specific alkaloids were then measured using HPLC/HRMS and unequivocally showed the presence of taxines in the blood.

The findings of the enquiry into the patient profile and/or the discovery of vegetable particles in the digestive tract may warrant testing for yew poisoning. The absence of conclusive signs warranting testing may miss such poisonings. For this reason, it may be pertinent to test for these toxins in cases of unexplained deaths.

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### Fatal Poisoning, *Taxus Baccata*, Suicide

## K3 Phencyclidine (PCP) Prevalence and Demographics in Driving While Intoxicated/Driving Under the Influence of Drugs (DWI/DUID) and Postmortem Casework in Harris County, Texas, 2013–2018

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**Learning Overview:** After attending this presentation, attendees will have learned about the prevalence of PCP in DWI/DUID and postmortem casework analyzed in Harris County, TX, from the years 2013 through 2018. Attendees will learn about the demographic profiles and blood concentrations of PCP observed in these cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing demographic and toxicological information for PCP use observed in different types of casework in Harris County over a six-year period. This research provides information regarding trends in PCP use in the greater Houston area as compared to driver-related cases previously reported for the city of Houston.<sup>1</sup> The objective of this research was to also recognize any demographic and toxicological differences in DUID and postmortem casework associated with PCP use in Harris County.

In 2018, Harris County remained the third-most populous county in the nation, with a population exceeding 4.6 million people. For DWI/DUID casework, the Harris County Institute of Forensic Sciences (HCIFS) serves various unincorporated areas of Harris County, the Harris County Sheriff's Office, the Harris County Constable, and multiple incorporated municipal police departments, excluding the city of Houston, which is policed by the Houston Police Department and serviced by the Houston Forensic Science Center. For postmortem casework, the HCIFS medical examiner crime laboratory performs testing for all of Harris County, including the city of Houston.

Between the years 2013–2018, HCIFS received 15,922 DWI/DUID cases. Of these cases, 5,156 had blood ethanol concentrations of < 0.17g/100mL (January 2013–June 2017) or < 0.10g/100mL (June 2017–December 2018) and were therefore subjected to drug testing. Cases with blood ethanol concentrations above those cutoffs were not analyzed for drugs. There were 3,676 drug-positive cases, and PCP was quantified in blood for 319 cases (2.0% of all DWI/DUID cases; 6.9% drug-tested cases; 8.7% of drug-positive cases). Concentrations ranged from 4.7–187µg/L with mean and median concentrations of 49 and 46µg/L, respectively. Ages of offenders ranged from 20–57 years, with mean and median age of 35 years. Females accounted for 19.12% of the cases, and males were identified in 80.88% of the cases. Races/ethnicities as identified by the submitting officer were: 78.37% Black, 18.50% White, 1.88% Unknown, 0.94% Hispanic, and 0.31% Asian. As with the data for the city of Houston, the average age of the offender was in their 30s, and Black males comprised the majority of PCP-positive impaired drivers.

During this same period, 229 postmortem cases were positive for PCP in blood. Mean (median, range) concentrations were 249µg/L (184, 3.9–5,280µg/L). Ages of decedents ranged from 19–63 years, with mean and median ages of 36 and 35 years, respectively. The demographic cohorts were 17.03% female, 82.97% male, 83.41% Black, 9.17% Hispanic, 5.68% White, and 1.75% Asian. Of these 229 cases, PCP-toxicity was identified in 87 cases as a primary contributor to cause of death, alone or in combination with other drugs or underlying health conditions. Mean (median, range) concentrations were 282µg/L (140, 11–5,280µg/L). These decedents were 24.14% female, 75.86% male, 82.76% Black, 6.90% Hispanic, 8.05% White, and 2.30% Asian. For the remaining 142 cases where PCP toxicity was not identified as a contributor, mean (median, range) concentrations were 230µg/L (192, 3.9–828µg/L). As with DUID casework, decedents were predominantly Black males, with most individuals aged in their 30s.

Demographic data and blood-PCP concentrations are comparable with those previously reported in DWI/DUID casework for the city of Houston (7–180µg/L) and in the literature.<sup>1–3</sup> However, fatal blood concentrations of PCP in the literature are highly variable and have been reported to range from 300–25,000µg/L.<sup>3</sup> The observed concentration ranges were lower in casework at HCIFS, further demonstrating that these fatal ranges have potential to overlap with each other and with what is observed in impaired driving casework. Although concentration ranges can be difficult to define, this research indicates that in Harris County there are demographic trends showing that most users are Black males and the average user will be in their mid-30s. Recognizing these population trends for PCP use can help implement and improve upon programs designed to reduce substance abuse in high-risk areas.

### Reference(s):

1. Lee and Stout. Toxicological and demographic profiles of phencyclidine-impaired driving cases in Houston. *J Anal Toxicol* 2020;44:499-503
2. Levine. *Principles of Forensic Toxicology*, 4<sup>th</sup> ed. AACC Press, 2015; 372-280
3. Baselt. *Disposition of Toxic Drugs and Chemicals in Man*, 11<sup>th</sup> ed. Biomedical Publications, 2017; 1680-1681

### PCP, Toxicology, Blood

## K4 The Return on Investment From Reducing Turnaround Time for Processing Driving Under the Influence (DUI) Drug Cases

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**Learning Overview:** After attending this presentation, attendees will be able to effectively plan for personnel needs as external forces alter the demands for toxicological analysis in forensic crime laboratories. That planning includes anticipating staffing increases and training to support increased demands for services. This presentation offers measures on the societal benefits from the identification and anticipation of emerging trends.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing laboratory management with an independent assessment of the personnel support required to meet emerging demands for analysis. Laboratory directors will have quantifiable measures of societal gains to support appeals to funding bodies for increased resources to meet demands for services.

From the opioid crisis to legalization of marijuana to the continuing evolution in the abuse of drugs, the societal costs from substance abuse are extensive. This includes societal costs from treatment, health care, lost productivity, death, and costs to the justice system. The justice system costs range from policing to the crime laboratories to jails, courts, and prisons.

This presentation examines the costs associated with the evolution of drug use as reflected in the growth of Driving Under the Influence of Drugs (DUID) cases. The societal return on investment is presented from the allocation of additional resources toward the problem of drugged driving, and the bottleneck in the justice system from too few resources devoted to emerging drug use in society, both legal and illegal.

As a seemingly invisible participant in the justice system's efforts to deal with the constant change, the forensic crime laboratory has been inundated with casework related to evolving drug use from drug chemistry, toxicology antemortem and postmortem. Unfortunately, the resources to deal with this growing social problem have failed to materialize. Could the resource demands be foreseen? Consider, for example, Washington state. The legalization of marijuana was expected to generate over \$300 million annually in tax revenues, and that has come to fruition. Yet, additional permanent funding was not planned for the forensic crime laboratory, and the backlog in toxicology has exceeded 6,000 cases. While some emergency funding has been allocated, the permanent investment in staffing is lacking.

As other states consider the passage of medical or recreational use of marijuana, planning for the demands upon the forensic laboratory should be included as an associated cost with resources dedicated for testing. Illumination of the societal costs will assist in that planning.

The costs from DUID go well beyond marijuana use. A recent analysis of the rise in Phencyclidine (PCP) use in Houston demonstrates the need to react quickly and provide the needed funding to the laboratory.<sup>1</sup>

As highlighted by a 2017 White House report, the opioid crisis has resulted in an annual societal cost that exceeds two percent of gross domestic product. Reaction to deal with the crisis has been slow and the costs mount. And, when efforts are directed toward a specific form of abuse, such as prescription pain killers, the drug of choice changes to black tar heroin to fentanyl to other synthetic opioids. This presentation centers on the return on investment to anticipating and reacting quickly to change and avoiding severe down-the-road costs.

This analysis will assist policymakers for proactive planning and reactive support to the justice system. There is a considerable amount of literature and data available. Data from the National Highway Traffic Safety Administration, insurance industry, and project FORESIGHT will be used to estimate the costs of delay in processing DUID cases. This demonstrates the statistical technique to estimate the costs of delayed processing and compares the associated opportunity cost with the cost of additional staffing targeted toward alternative turnaround times in the laboratory.<sup>2</sup> Together, these estimates provide the return of investment metric for effective demonstration to policymakers for the need for additional funding.

### Reference(s):

1. Lee, D., and Stout, P. (2020). Toxicological and demographic profiles of phencyclidine-impaired driving cases in Houston. *Journal of Analytical Toxicology*, 244, 499-503.
2. Aldy, J.E. and Viscusi, W.K. (2008). Adjusting the Value of a Statistical Life for Age and Cohort Effects. *The Review of Economics and Statistics*, 90(3), 573-581.

### Toxicology, Staffing, Management

## K5 A Validated Method for the Quantitative Determination of Isotonitazene 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 a validated method for the detection and quantification of isotonitazene and its metabolites in hair by 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 isotonitazene and its metabolites in hair.

**Introduction:** Isotonitazene is considered as an emerging New Psychoactive Substance (NPS) being sold as a designer drug. Preclinical pharmacology studies show that isotonitazene has the pharmacological profile similar to that of the potent synthetic opioid etonitazene, a Schedule I controlled substance. Because of the pharmacological similarities of isotonitazene to etonitazene, the use of isotonitazene presents a high risk of abuse and may negatively affect users and communities. According to the Federal Register (2020), abuse potential of isotonitazene is very high and no treatment is available in the United States. Pharmacological data suggest that isotonitazene has a potency similar to or greater than fentanyl and many fentanyl analogs. It is considered to be 2.5 times more potent than hydromorphone. Detection times of drugs and metabolites in hair are much longer than in urine, blood, and oral fluid. Blood and urine are utilized mainly in acute ingestions of isotonitazene, while hair is ideal for detection in chronic abuse. Therefore, hair is an ideal matrix for the detection of isotonitazene and metabolites if the tests are performed weeks after intake.

**Methods:** Recently, an LC/MS/MS method was developed for the detection, identification, and quantification of isotonitazene and its metabolites, 5 aminoisotonitazene and 4- hydroxy nitazene, in hair samples. Briefly, the method involved extraction of drugs and metabolites from hair using acidic methanol. The dried extracts, after reconstitution with organic/aqueous solvents mixture, were injected onto an Agilent® 6460 Triple Quadrupole (QQQ) LC/MS/MS in positive ionization mode. Separation was achieved on an Agilent® ZORBAX® Eclipse XDB-C18 column (4.6 x 50mm, 1.8µm) with a flow rate of 0.4mL/min of 5mM ammonium formate:methanol (9:1) (A) and 0.1% formic acid in methanol (B) mobile phase under gradient conditions. Sample preparation involves washing of hair with aqueous and organic solvents to remove the external contamination followed by incubation with 2mL of acidic methanol at 60°C.

**Results:** Good linearity and reproducibility were obtained for isotonitazene and metabolites with a coefficient of determination  $R^2 > 0.99$ . The linear range of the assay was 100–2,000pg/mg. Limits Of Quantification (LOQ) ranged from 1pg/mg to 5pg/mg for isotonitazene and its metabolites. Excellent imprecision and accuracy were achieved (CV <15%) for all compounds.

### Isotonitazene, LC/MS/MS, Drug Testing

## K6 A Suicidal Sodium Nitrite/Nitrate Ingestion Outbreak in Houston, Texas

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**Learning Overview:** After attending this presentation, attendees will understand the concern for easily available chemicals that can be used as toxins and the importance of Methemoglobin (MetHb) saturation in postmortem cases involving sodium nitrite and sodium nitrate ingestion. Six suicidal cases in eight months from late 2019 to early 2020 will be presented with relevant case findings and toxicology results, including MetHb saturation. The postmortem MetHb saturation found in these cases will be compared to published antemortem and postmortem MetHb saturation in fatal cases involving methemoglobinemia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to commonly used chemicals that can be used as toxins in postmortem cases. Sodium nitrite and sodium nitrate are readily accessible chemicals that are used in different manufacturing and industrial purposes, such as food preservation and fertilizers. While relatively harmless at low concentrations present in food, at higher concentrations, nitrite leads to oxidation of the iron in hemoglobin, converting it to inactive MetHb.<sup>1</sup> Nitrate can cause the same oxidation after being reduced to nitrite by bacteria in the body.<sup>1</sup> Low (<5%) saturation of MetHb is considered normal. When saturation reaches or exceeds ~20%, cyanosis, headaches, vomiting, loss of consciousness, and other toxic effects can occur, and >70% saturation is considered to be lethal.<sup>2</sup>

As nitrite and nitrate are not commonly investigated in routine toxicology testing, exposure, and toxicity to these agents are often limited to autopsy findings and indirect toxicology testing such as MetHb saturation. Being able to use MetHb saturation as a tool to aid in determining cause of death is important in the absence of other significant toxicological results. As most published nitrite/nitrate exposure cases typically provide antemortem MetHb levels, providing postmortem MetHb saturation levels in suicidal nitrite/nitrate exposure cases can assist in interpretation.

**Methods:** Postmortem blood specimens for the six cases were sent to NMS Labs for MetHb saturation testing by spectrophotometry. Blood sources for these cases included heart ( $n=1$ ), iliac ( $n=1$ ), and femoral ( $n=4$ ) that were collected in lavender-top tubes. Additional in-house testing varied for each case based on the case circumstances and pathologist requests, but all cases did include testing for ethanol, methanol, isopropanol, and acetone along with a 10-panel enzyme-linked immunosorbent assay screen.

**Results:** The six cases included four males and two females that ranged in age from 17 to 29 years old. MetHb saturation ranged from 16–47% with no other significant toxicological findings other than one case where etizolam was identified. Autopsy findings included blue-gray cutaneous discoloration and dark brown discoloration of the blood and various tissues. In six other published fatal methemoglobinemia cases, MetHb saturation was reported in postmortem blood for five cases and repeated antemortem blood in one case.<sup>3-6</sup> The postmortem saturation in the published cases ranged from 23–95%. For the case with repeated antemortem levels, the initial MetHb saturation was reported as 70%, followed by 30% post-treatment with methylene blue, and finally dropped to 18% after a transfusion before death occurred. In non-fatal cases, the MetHb saturation has been reported as low as 19% and as high as 94% after nitrite exposure.<sup>7,8</sup>

**Discussion/Conclusion:** Due to the ease of access of nitrite and nitrate containing compounds, concerns for their exposure cannot be ignored in toxicology cases. It is clear from fatal and non-fatal nitrite exposure cases that the MetHb saturation varies greatly and there is significant overlap in the MetHb saturation and outcome. The antemortem MetHb saturation levels that are used to establish risk also do not appear to be relevant in postmortem cases, and more information regarding MetHb in postmortem cases involving methemoglobinemia and nitrite/nitrate exposure would be useful to better understand an expected range to assist in interpretation.

### Reference(s):

1. Baselt, Randall C. Nitrite. *Disposition of Toxic Drugs and Chemicals in Man*, 11<sup>th</sup> ed. (Seal Beach, CA: Biomedical Publications, 2017), 1539-1541.
2. Hall, Alan H., Kulig, Kenneth W., and Barry H. Rumack. Drug- and Chemical-Induced Methaemoglobinaemia Clinical Features and Management. *Medical Toxicology* 1 (1986): 253-260.
3. Harris, John C., Rumack, Barry H., Peterson, Robert G., and Brian M. McGuire. Methemoglobinemia Resulting from Absorption of Nitrates. *Journal of the American Medical Association*, 242 (1979): 2869-2871.
4. W. J. Gowans. Fatal methaemoglobinaemia in a dental nurse. A case of sodium nitrite poisoning. *British Journal of General Practice* 40 (1990): 470-471.
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8. Edwards, R.J. and J. Ujma. Case Report: Extreme methaemoglobinaemia secondary to recreational use of amyl nitrite. *Journal of Accident and Emergency Medicine* 12 (1995): 138-142.

### Methemoglobin, Sodium Nitrite/Sodium Nitrate, Suicide

## K7 A Suspected Case of Attempted Homicide by Rodenticides Administration: How Hair Analysis Can Help Us in Solving the Mystery

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**Learning Overview:** After attending this presentation, attendees will recognize the importance of applying segmental hair analysis in some cases of suspected intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting how forensic hair analysis could be pivotal for the chronology of exposure to toxins.

A 72-year-old man presented to the local hospital with hematuria and abdominal pain. Abnormal coagulation parameters (Prothrombin Time-International Normalized Ratio [PT-INR] between 16.14 and 19.07) were measured. During hospitalization, his PT-INR result was hard to stabilize, ranging from 2.72 to 12.18, despite numerous vitamin K infusions. Eventually, his blood tests resulted positive for anticoagulant rodenticides, so the case was reported to the public prosecutor's office. The man had been hospitalized for similar symptoms many times in the previous months. The patient's hair sample was taken 19 days after hospitalization. Analyses of blood, collected during hospitalization, hair, and material seized at the man's house were carried out.

**Methods:** 500 $\mu$ L blood samples were acidified with 1mL HCl (0.1M) and extracted using a Liquid-Liquid Extraction (LLE) procedure. Hair samples were extracted in 1mL methanol; then, the organic solvent was evaporated and reconstituted in 100 $\mu$ L methanol. Warfarin-D5 was used as an internal standard for both procedures. The seized material was diluted 1:10 with methanol and directly injected in the Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) system. A C18 column (100 $\times$ 2. mm i.d., 2.6 $\mu$ m particle size) was used in reversed phases, the mobile phases consisted of 0.1% formic acid in bidistilled water (phase A) and 0.1% formic acid in acetonitrile (phase B). The triple quadrupole was operated in Multiple Reaction Monitoring (MRM) negative mode. The following transitions were selected for identification and quantification:  $m/z$  -443.1/-135.0 and -443.1/-293.4 for difenacoum (declustering potential: -98V; collision energy: -43eV);  $m/z$  -541.4/-161.1 and -541.1/-117.1 for flocoumafen (declustering potential: -55V; collision energy: -40eV);  $m/z$  -312./-255.2 for warfarin-D5 (declustering potential: -55V; collision energy: -30eV).

**Results and Discussion:** Blood positivity was confirmed in all samples, with difenacoum at a concentration between 17.0 and 51.9ng/mL, while flocoumafen was found between 23.0 and 140.0ng/mL. In the proximal hair segment (1cm), difenacoum was detected in traces (Limit Of Quantitation [LOQ]=5pg/mg) while flocoumafen was measured at a concentration of 19.0pg/mg; in the intermediate segments (1-2 and 2-3cm), both difenacoum and flocoumafen were absent; in the distal segment (3-5cm), difenacoum was found in significant amounts (140.0pg/mg), while flocoumafen was lacking. Of all the seized material, only two contained rodenticides: a specimen similar to a red cereal (which contained difenacoum) and a number of blue pills (which contained flocoumafen) found at the man's house.

**Conclusions:** The presence of difenacoum and flocoumafen both in blood and in hair samples indicates that the man was poisoned with both of the molecules in the days immediately preceding his hospitalization, while there was a complete lack of exposure in the previous months. Yet, difenacoum was administered, probably multiple times, at least four months before hospitalization. Per research, there are few cases reported in literature about detection of rodenticides in keratin matrices; moreover, this case, for the first time, proved that segmental hair analysis of anticoagulant agents could represent important additional information for the interpretation of the case.

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### Rodenticides, Hair Testing, Intoxication



## K8 A Veterinary Forensics Review of Pesticide Toxicity in Canines

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**Learning Overview:** After attending this presentation, attendees will be familiar with pesticides that may affect canine health and safety, toxicological and clinical parameters following incidents of canine pesticide intoxication, and the present state of knowledge/awareness held by veterinarians and canine care providers relative to pesticide intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a knowledge gap and dearth in availability of consolidated tools. As such, the field of forensics will gain merged data on the toxicity of varying pesticides in dogs for use in related investigations, diagnoses, treatment, toxicological analysis, and pesticide management. This will especially benefit veterinary practices, forensic toxicologists, forensic veterinarians, pesticide users, pet dogs (and their owners), and dogs working in various disciplines ranging from detection to agricultural sectors.

Proximity to humans, a curious nature, and (where applicable) their assigned duties, can all predispose dogs to pesticide intoxication. In this context, “pesticides” describes chemicals manufactured for agricultural/gardening pest control or health purposes. This definition includes rodenticides, insecticides, fungicides, nematicides, and veterinary medications, such as heartworm or flea/tick treatments. Veterinary products, insecticides, rodenticides, and garden products accounted for some of the top toxins that affected pets in 2017.<sup>1</sup> While data exists on doses of pesticides toxic to dogs, such information remains disparate; a corresponding consolidation of chemical analysis of pesticides, and diagnoses/treatment of pesticide intoxication related to dogs, is not yet available in one place. An extensive literature review and data mining were conducted to evaluate the existing information and collate it to facilitate interpretation and access.

Toxicological and chemical variables of varying pesticides are provided to aid in evaluating toxic doses and help steer analytical screening of pesticides to confirm or rule out involvement in cases of canine toxicosis. These data were mined from previous pharmacology studies and pesticide reviews conducted by regulatory agencies and industrial pesticide manufacturers. Clinical data are provided to educate on toxicosis symptoms and treatments associated with varying pesticides and routes of exposure. This was gathered from case studies and data provided by veterinary practices.

A survey was conducted to glean information concerning pesticide toxicity in canines directly from practicing veterinarians across the United States. Over 1,000 surveys were sent through email or an anonymous link, and 57 responses were obtained. Results of the survey provide insight in the prevalence of pesticide intoxication in dogs and awareness of veterinarians to its existence, and to existing veterinary capacity in place to identify, address, and prevent such cases. For example, while 66% of respondents have someone working in the practice that has been educated on the topic of pesticides exposure in companion animals and 75% have a dependable reference source for identification/treatment of pesticide intoxication, 46% are not aware of ways to report these incidents and 58% do not report incidents. Further findings from the survey will be presented, with future recommendations based on the consolidated datasets as they relate to the survey findings.

### Reference(s):

<sup>1</sup>. Top 10 Animal Toxins of 2017. *ASPCAPro*, last modified March 29, 2018, <https://www.aspcapro.org/resource/top-10-animal-toxins-2017>

### Pesticide, Toxicity, Canine

## K9 Measurement Uncertainty (MU): A Novel Quality Assurance Use in Forensic Toxicology for Internal Proficiency Testing

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**Learning Overview:** The goal of this presentation is to show the process of using MU to determine acceptability of test performance by using illustrative examples based upon true proficiency test samples and root cause analysis outcomes to demonstrate the utility of this approach.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that MU established for an analytical method can be utilized for grading proficiency results.

**Background/Introduction:** Every measurement made has inherent uncertainty associated with it. MU allows an analytical result to be presented along with an associated interval that incorporates all identified error sources in the measurement process. The provided interval is the result of the accumulation of these errors and expansion of the final uncertainty to a predefined level of probability that the true value lies within this interval. In forensic toxicology, MU is most important for those cases where legal consequences are determined based upon *per se* laws and the reported analytical measurements. The utility of MU, however, is not limited to this purpose. Proficiency Testing (PT) is an essential component to any laboratory's Quality Assurance program, and objective measures of performance are needed. Ongoing PT provides assurance that reported data are accurate and that any issues are identified and appropriately addressed. While most PT programs grade the results, there may be instances where the program requires the laboratory to determine their own grading criteria for self-assessment purposes. While one common way to accomplish this task is to determine if the reported concentration (e.g., 180ng/mL) is within a certain percentage (e.g., +/- 20%) of the target concentration (e.g., 200ng/mL), this study, when faced with this circumstance, opted to use the MU established for the analytical method to grade the tested proficiency samples. This approach is applicable as it not only allows for assessment of the reported result, but also considers the applied MU range.

**Methods:** This approach was accomplished by taking the Grand Mean provided by the PT program for each positive sample and determining if this value was encompassed by the reported concentration and its associated MU interval for each internal result. Proficiency results were graded and, when applicable, Root Cause Analysis performed. Measures to address any quality performance issues were then implemented based upon the totality of the investigation.

**Results:** Results from one PT with use of this grading approach are shown in the following table:

Sample No.	Grand Mean (g/100mL)	Result Range (Reported Result + MU) (g/100mL)	Acceptable Result Range (g/100mL)	Proficiency Outcome
1	0.0543	0.048 ± 0.002	0.046 – 0.050	Investigate
2	0.3117	0.304 ± 0.009	0.295 – 0.313	Pass

In this instance, for Sample 1, the percent difference between the reported concentration (0.048g/100 mL) and the Grand Mean (0.0543g/100 mL) is -12%, which may be marked as Passing. Using the Acceptable Result Range, however, a low bias is noted and Sample 1 is marked as Investigate and an appropriate Root Cause Analysis can be initiated.

**Conclusion/Discussion:** Verification of result accuracy and the applied MU range should be used, where appropriate, to evaluate PT results. Furthermore, it is best practice due to *per se* laws that the utility of the MU be checked on a routine basis with PT being an evidenced-based way to do so. This approach provides a routine mechanism for staff to understand the value and limitations of MU as well as the associated explanation of its use.

### Uncertainty, Proficiency, Quality

## K10 False Positive Emit II Plus Ecstasy Assay Due to Bupropion

*Maja Jelena Cop\*, Zagreb 10000, CROATIA; Sandra Mudric, Zagreb, Croatia, CROATIA*

**Learning Overview:** The goal of this presentation is to learn about the causes of false positive screening toxicology tests and how to deal with such results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge about false positive screening results in toxicology.

This study describes a forensic toxicology case in which evaluating the driving under influence of drugs was required. Blood and urine samples were taken from an injured driver involved in a car accident to perform drug analysis.

Toxicological analysis started with preliminary screening of urine using a Syva VIVA-E analyzer and Emit II Plus Assays for barbiturates, benzodiazepines, Tetrahydrocannabinol (THC), cocaine, methadone, opiates, ecstasy, and amphetamines. Screening tests showed that the urine was positive for benzodiazepines, THC, cocaine, and ecstasy. Blood and urine were then analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The results of confirmation methods showed the presence of diazepam, midazolam, alprazolam, THC and its metabolites, cocaine and its metabolites, and the antidepressant drug bupropion. But, ecstasy or other drugs which cross-react to 3,4-Methylenedioxymethamphetamine (MDMA) EMIT II Plus Assays were not detected at all. Since it is known from experience that benzodiazepines, THC, and cocaine would not react positively to the MDMA Assay, it was assumed that it might be due to bupropion. However, in the EMIT Cross-Reactivity list, bupropion is listed in the list of drugs structurally related to MDMA that react positively to the MDMA Assay only in non-clinically significant concentrations, which means in much higher concentrations than normally seen in patients taking these drugs.<sup>1</sup> According to the MDMA Cross-Reactivity list, the concentration of bupropion that would react positively to the MDMA Assay is 4,400µg/mL.

In this case, the quantitative analysis of bupropion in urine was not performed due to a lack of a deuterated standard. Nevertheless, considering the results of the qualitative GC/MS analysis and all the available data regarding the case, it was possible to assume that the driver consumed the usual therapeutic dose of bupropion and that the bupropion concentration in his urine was common.

Since the screening methods are fast, inexpensive, and do not require complicated sample preparation, due to a large number of forensic toxicology cases, they are used as a triage: if the result of screening is positive to any listed drugs, samples are analyzed by GC/MS and/or LC/MS/MS, and samples negative on screening are not analyzed any further. Consequently, it is clear that the reliability of the screening method is of great importance in forensic toxicology, and a lot of attention has been paid to it, especially when it comes to unusual results, as in this case.

Therefore, nine solutions of bupropion standard with concentrations from 15µg/mL–2,000µg/mL were tested on EMIT II Plus MDMA Assay to determine the cut-off concentration of bupropion that gives a positive reaction. The result showed that cut-off was 150µg/mL of bupropion, which is about 30 times lower than the concentration given in the MDMA Cross-Reactivity list.<sup>1</sup>

### Reference(s):

- <sup>1.</sup> *EMIT Drugs-of-Abuse Urine Assays Cross-Reactivity List.*  
[https://kids.wakemed.org/documents/pathology/EMIT\\_Drugs\\_of\\_Abuse\\_Cross\\_Reactivity\\_List.pdf](https://kids.wakemed.org/documents/pathology/EMIT_Drugs_of_Abuse_Cross_Reactivity_List.pdf)

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### EMIT Ecstasy Assay, Bupropion, Cross Reactivity

## K11 Expanding Frontiers in Postmortem Toxicology: Drug Tracing in Different Postmortem Matrices During Human Decomposition Using Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS)

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of a highly sensitive UHPLC-HRMS approach for detecting drugs postmortem throughout soft tissue decomposition.

**Impact on the Forensic Science Community:** This presentation will positively impact the forensic science community by revealing novel aspects of inter-individual toxicological differences in serum, fly larvae, decomposition fluid, and soil from human donors during the early and late stages of decomposition.

Decomposition rates of donors placed in identical environmental conditions appear to be verifiably different, therefore the focus of this study is on intrinsic drivers of human decay that may have the potential to alter decomposition trajectories.<sup>1,2</sup> The ultimate goal is to elucidate how drugs impact human decomposition rates and, ultimately, Postmortem Interval (PMI) estimation. Entomotoxicological experiments by Goff et al. revealed an impaired insect development for certain drugs of abuse; however, thus far it is completely unknown how drugs, particularly prescription and end-of-life medications, impact patterns of human decomposition.<sup>3</sup> This presentation provides insights on the analysis of drugs in several compartments of the highly dynamic postmortem biochemical environment from decomposing bodies through primary microbial and insect decomposers.

This research was conducted on human donors, obtained through the Body Donation Program of the Forensic Anthropology Center (FAC), and decomposed in an outdoor forested setting at the University of Tennessee Anthropology Research Facility (ARF) in Knoxville, TN. Serum, soil, fly larvae, and decomposition fluid samples were obtained from human subjects at various time points during soft tissue decomposition, flash frozen and analyzed for their toxicological composition. Decomposition fluid was collected at various anatomical positions from around the body; larvae were collected at each instar as well as during the post-feeding stage. 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. Termination of sampling occurred after the bodies completed active decay.

Aliquots of all specimens were extracted using a procedure adapted from Lu et al.<sup>4</sup> Samples were injected onto a Raptor Biphenyl column (100mm x 2.1mm) with 2.7- $\mu$ m particle size in combination with a guard column (5mm x 2.1mm, 2.7- $\mu$ m). Afterward, the eluent was introduced into a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap via positive electrospray ionization. A full scan Mass Spectrometry (MS) analysis (70–1,050m/z) was performed, and samples analyzed for 12 minutes with a resolution of 140,000. Commercial drug standard stocks of 15 drugs from major drug classes were purchased for quantitative analyses. Spectral features were manually selected based on mass accuracy ( $\pm$  5ppm mass tolerance) and retention times ( $\leq$  2min) using an adapted drug database from Restek®.<sup>5</sup>

Initial toxicological screenings of nine donors revealed that both parent drugs and their drug metabolites are traceable postmortem over time from the initial serum sample to later samples of decomposition fluid and primary decomposers, to ultimately the local soil matrix. Moreover, it was possible to assign each donor a unique toxicological profile, which showed time-dependent changes and variability in detected drug intensities for each of the four analyzed matrices throughout the decomposition period. The current results show, first, the ability and, second, the unexplored potential of drug detection in a series of different matrices after death. Furthermore, they seem to reveal first signs of a possible direct relationship of drug-induced effects on decomposer physiology. Ongoing “big data” analysis will combine toxicological screening with metabolomics and lipidomics data sets to identify potential biochemical biomarkers of decomposition and create an overall picture of how postmortem metabolite signatures relate to perimortem toxicological loadings.

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### Forensic Chemistry, Human Decomposition, Drug Analysis

## K12 An Analysis of Tramadol and Its Metabolites in Rat Skeletal Tissues Following Acute and Repeated Dose Patterns Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)

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**Learning Overview:** The goal of this presentation is to demonstrate the utility of LC coupled with MS/MS for the semi-quantitative analysis of tramadol and four of its metabolites in animal models that underwent different dosing patterns. Additionally, this presentation seeks to showcase the efficacy of assigning a numerical value representing the frequency of significant differences in order to simplify the results obtained in works where a large number of statistical tests were performed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of skeletal tissues as a suitable alternative matrix for toxicological analysis. Furthermore, this presentation will demonstrate the importance of a multifaceted approach to toxicological analysis, including appropriate statistical modeling to differentiate between various patterns of drug use.

The use of skeletal elements for the viable analysis of drugs of abuse has seen increased prevalence in the past ten years. Advancements in the analytical methods used, including solid phase extraction and MS, have allowed for increased sensitivity and selectivity. Previous studies have focused on the influence of dose-death interval, microclimate, differential patterns of exposure, and the influence of body position. In this work, the opioid analgesic tramadol was investigated for its pharmacological behavior when administered as part of three dosage patterns to male Sprague Dawley rats. The three exposure patterns consisted of an acute low ( $n=4$ , 1 dose, 30mg/kg) group, a repeated high survived ( $n=5$ , 3 doses, 30mg/kg) group, and a repeated high overdosed group ( $n=11$ , 3 doses, 30mg/kg). Drug-free rats ( $n=4$ ) served as negative controls.

Following euthanasia by CO<sub>2</sub> asphyxiation, animals were decomposed to skeletons outdoors over the summer of 2019 in Sudbury, ON. Bones were sorted by animal and skeletal element (skull, vertebrae, ribs, pelvis, femur, tibia/fibula), then washed and ground to powder before undergoing dynamic methanolic extraction. Semi-quantitative analysis of tramadol and four of its metabolites—O-desmethyltramadol, N-desmethyltramadol, N,O-didesmethyltramadol, and tramadol N-oxide—was conducted using HPLC/MS/MS in positive ion mode. Analyte levels were expressed as a mass-normalized Response Ratio (RR/m) in order to account for the exact mass of bone used. Method validation for the analysis of tramadol and its metabolites was investigated in accordance with the Scientific Working Group of Toxicologists (SWGTOX) standards of practice, with all criteria except for dilution integrity successfully met at a limit of detection and limit of quantification of 1ng/mL. The effect of exposure pattern on analyte level and analyte level ratio was assessed using the Kruskal-Wallis test for significant differences ( $P < 0.05$ ). A total of 315 pairwise comparisons were performed to assess significant differences, with the ratio of tramadol to N-desmethyltramadol determined to be the metric most commonly able to identify these differences in 91% of tests. Additionally, the effect of skeletal element on analyte level and analyte level ratio was also assessed, with a total of 675 pairwise comparisons. Skeletal element was determined to be a significant factor in all cases.

These data suggest that both skeletal element and dose pattern are important measures to evaluate with respect to the analysis of drugs of abuse in bone tissues. Furthermore, different metrics, including analyte level and analyte level ratios, may be useful for discriminating between these different dosing patterns.

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### Tramadol, Tandem Mass Spectrometry, Skeletal Tissues

## K13 A Fatal Poisoning of Four Workers at a Farm: The Distribution of Hydrogen Sulfide (H<sub>2</sub>S) and Thiosulfate(TS) in Different Biological Matrices

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**Learning Overview:** After attending this presentation, attendees will have improved their knowledge about the principles of evaluation of distribution of H<sub>2</sub>S and TS in biological fluids and tissues in cadavers, together with the postmortem investigations and the circumstantial data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by analyzing an unwitnessed case of four victims of acute intoxication by H<sub>2</sub>S, in particular showing how the concurrent measurement of sulfide and TS concentrations are fundamental to better understand the circumstances of the deaths, the timing, and the real sequence of the events, according to onsite and postmortem investigation.

H<sub>2</sub>S is a gas produced during putrefaction and found in many industrial processes, representing a not unusual cause of fatal events in workplaces. Reported here are the toxicological investigations applied in an accidental poisoning by H<sub>2</sub>S inhalation involving four dairy farmers. The men were found dead in a pit connected to a manure lagoon.

An onsite investigation was immediately conducted. A manure spreader tank truck was parked near the pit. The corpses were partially submerged in the sewage, at the corners of the half-full pit, but their position did not help explain the possible dynamics of the events. The valve that allows the sewage to exit from the manure pool to the pit was open but did not work properly.

Autopsies were performed four days after death. Biological samples were collected into vials. Toxicological analyses of sulfide and its main metabolite, TS, were made using an extractive alkylation technique combined with Gas Chromatography/Mass Spectrometry (GC/MS).

Autopsies revealed multiorgan congestion and pulmonary edema. Manure was found inside airways of Subjects 1, 2, and 4.

Sulfide concentrations were cardiac blood: 0.5–3.0µg/mL; femoral blood: 0.5–1.2µg/mL; urine <0.1µg/mL; bile: <0.1–2.2g/mL; liver 2.8–8.3µg/g; lung: 5.0–9.4µg/g; brain: 2.7–13.9µg/g; spleen: 3.3–6.3µg/g; fat: <0.1–1.5µg/g; and muscle: 2.6–3.5µg/g. TS concentrations were: cardiac blood: 2.1–4.9µg/mL; femoral blood: 2.1–2.3µg/mL; bile: 2.5–4.4µg/mL; urine: <0.5–1.8µg/mL; liver <0.5–2.6µg/g; lung: 2.8–5.4µg/g; brain: <0.5–1.9µg/g; spleen: 1.2–2.9µg/g; muscle: <0.5–5.6µg/g; and fat: <0.5µg/g. Concentrations of sulfide and TS were consistent with values found in fatal cases of hydrogen sulfide poisoning.<sup>1-4</sup>

The cause of the death was assessed to be acute poisoning for all victims. Manure inhalation contributed to the death of Subjects 1, 2, and 4. Interestingly, non-homogeneous toxicological values were detected. Data interpretation assumes that Subject 3 was the first to enter the pit, probably to open the blocked valve. He died immediately after that, considering he had the highest concentration of sulfide in brain: it is indeed known that sulfide may cause rapid loss of consciousness and respiratory depression. One by one, the other farmers entered the pit in attempts to rescue the coworkers but were overwhelmed: they all died shortly after. Despite the rapid deaths, Subject 3 was the only one with TS detectable in urine (1.8µg/mL). This could be due to differences in metabolism of H<sub>2</sub>S or to a previous exposure.

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### H<sub>2</sub>S, Thiosulphate, Intoxication

## K14 The Development of a Simultaneous Separation and Identification Ultra Performance Liquid Chromatography/High-Performance Liquid Chromatography (UPLC/HPLC) Tandem Mass Spectrometry (MS/MS) Screening Method for Sulfur-Containing Fentanyl Analogs (SFA)

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**Learning Overview:** After attending this presentation, attendees will understand a screening method to separate and identify SFA and isomers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method capable of separating and identifying newly emerging SFA utilizing UPLC-MS/MS.

**Background/Introduction:** In February 2018, the Drug Enforcement Administration (DEA) released a statement of the emergency scheduling (Schedule 1) of all illicit fentanyl analogs not already regulated by the Controlled Substances Act due to an alarming increase in overdose deaths linked to synthetic opioids. Fentanyl analogs are pharmacologically similar to fentanyl, but often more potent. This increased potency can create problems with proper dosing of fentanyl analogs and can lead to untoward effects, including an increase in overdoses and deaths. These newly emerging fentanyl analogs often appear on the street as diverted research chemicals; therefore, their identification can be difficult. Given the increase in overdose deaths, an analytical method for identification and separation of these analogs is needed to help the clinical and forensic communities overcome this epidemic.

**Objective:** To develop a method for the simultaneous identification and separation of 12 SFA: cis-3-methyl thiofentanyl, trans-3-methyl thiofentanyl,  $\alpha$ -methyl Thiofentanyl,  $\beta$ -hydroxythioacetylfentanyl,  $\beta$ -hydroxythiofentanyl, sufentanil (metabolite: norsufentanil), tetrahydrothiophene fentanyl, thienyl fentanyl, thiofentanyl, thiophene fentanyl, and 13C6  $\beta$ -hydroxythiofentanyl.

**Methods:** To improve laboratory method transfer, the fentanyl analogs were evaluated on two LC/MS/MS systems: UPLC-MS/MS (Waters® ACQUITY® UPLC-TQs-micro) and HPLC-MS/MS (Shimadzu® LC20AD XR coupled to AB SCIEX™ triple quadrupole 5500). In order to optimize these systems and identify ion transitions, the 24 transitions with their respective declustering potential and collision energy were determined using Electrospray Ionization (ESI) via manual infusion. Table 1 contains the ion transitions identified for identification. UPLC-MS/MS and HPLC-MS/MS conditions such as initial aqueous phase, organic phase, and gradient, as well as column chemistry were adjusted to achieve optimal separation and identification of the SFA. Organic phases evaluated were formic acid in acetonitrile and methanol; the aqueous phase for all analyses was formic acid in LC/MS water. The mobile gradients evaluated were curved, linear, and step type. Chromatography conditions were evaluated using LC and UPLC columns: Pentafluorophenylpropyl (PFPP), biphenyl, and several C18 columns, including CSH C18 and BEH C18, of variable lengths and end caps. Selectra PFPP 100 x 2.1mm 3 $\mu$ m; Restek® Ultra Biphenyl 50 x 2.1mm 3 $\mu$ m; Phenomenex® Kinetex® 100 x 3.0mm 2.6 $\mu$ m; UCT Selectra® C18 100 x 4.6mm 3 $\mu$ m; Waters® X-Select® CSH C18 50 x 2.1mm 2.5 $\mu$ m; Waters® ACQUITY® UPLC BEH C18 50 x 2.1mm 1.7 $\mu$ m.

**Results:** The Waters® X-Select® CSH C18 column resulted in the most chromatographic separation of the 12 SFA of the columns evaluated on the Shimadzu® LC coupled AB SCIEX™-5500 triple quad. The Phenomenex® Kinetex® biphenyl column resulted in comparable resolution to the CSH C18, the advantage of the CSH C18 is the partial spectral separation of the methylthiofentanyl isobars using one of the common transitions. The Water's® ACQUITY® UPLC BEH C18 resulted in the best chromatographic separation of the 12 SFA on the Water's® UPLC-MS/MS. A linear organic phase gradient 5% to 95% on the BEH C18 and 15% to 95% on the CSH C18 led to all compounds eluting after the solvent front with narrow, tall peaks and spectral separation of isomeric sulfur containing fentanyl analogs.

**Conclusion:** The BEH C18 had better overall chromatographic resolution: the BEH C18 uses a sub-2-micron particle size, which requires a UPLC system (>10,000psi). The CSH C18 had sufficient chromatographic resolution to be used on an HPLC system. One aspect of this study was to chromatographically separate and qualitatively identify the SFA; however, the cis and trans isomers of 3-methylthiofentanyl were not sufficiently resolved chromatographically to be able to identify them as separate analogs.

**Acknowledgments:** United States Department of Health and Human Services Centers for Disease Control and Prevention for their Traceable Opioid Material\* Kits (TOM Kits) and the National Institute of Health Grant (P30DA033934).

Table 1 identifies the transitions utilized for the identification of the 12 analogs on both the UPLC-MS/MS and AB SCIEX™-5500 triple quad MS.

SFA	Parent $m/z$	Transition 1 ( $m/z$ )	Transition 2 ( $m/z$ )
$\alpha$ -Methyl Thiofentanyl	357	125	259
$\beta$ -Hydroxythioacetylfentanyl	345	192	327
$\beta$ -Hydroxythiofentanyl	359	146	192
cis-3-Methyl Thiofentanyl	357	111	208
Norsufentanil	277	184	245
Sufentanil	387	238	355
Tetrahydrothiophene Fentanyl	395	105	188
Thienyl Fentanyl	329	97	180
Thiofentanyl	343	111	194
Thiophene Fentanyl	391	188	105
trans-3-methyl Thiofentanyl	357	209	259
13C6 $\beta$ OH Thiofentanyl	365	152	192

### Thiofentanyl, UPLC/MS/MS, Screening Method

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## K15 New Perspectives in Postmortem Diagnosis of Acute Heroin Abuse

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**Learning Overview:** The goal of this presentation is to provide an overview of the methods useful for the postmortem diagnosis of heroin abuse, with particular reference to immunohistochemistry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the availability of anti-heroin antibodies is able to take care of all situations in which there is no possibility of carrying out toxicological analyses.

Heroin, a semi-synthetic opioid drug synthesized from morphine, is the 3,6-diacetyl ester of morphine (diacetylmorphine). It is 2–3 times more potent than morphine, and the estimated minimum lethal dose is 100–200mg, but users may be able to tolerate up to 10 times as much. Compared to morphine, heroin is a more lipophilic compound and crosses the blood-brain barrier within 15–20 seconds and achieves relatively high brain levels; 68% of an intravenous dose is absorbed into the brain.

Heroin related-death can occur from: (1) absolute acute poisoning (intake of a quantity of heroin superior, in an absolute sense, to any tolerance); (2) acute intoxication related to tolerance (intake of a quantity of heroin greater than the degree of tolerance of the subject); and (3) first injection death (taking a lethal dose in a non-drug addict).

Toxicology data is certainly of greater importance for the diagnosis of heroin-related death, but, frequently, the conclusion of a heroin-related death is based upon a combination of scene investigation, physical examination of body, and the autopsy, as well as histological and toxicological findings. Postmortem diagnosis of heroin-related death, especially in the forensic field, could be a problem when it is not possible to perform toxicological data because blood or/and urine are absent.

The goals of this study are to evaluate the correlation between the concentration of heroin in the blood, bile, and urine in heroin-related death and an immunohistochemical study of heroin antibodies.

The toxicological data and the autopsy records of the 200 autopsies of drug related-deaths performed at the Departments of Forensic Pathology of the University of Rome over the period 2014–2020 were evaluated, and 14 cases of heroin-related deaths were selected (12 men, 2 women, mean age 44.4 years). The postmortem delay interval was  $\leq 36$ h in each case. Cases with toxicological data positive only for heroin and negative for other drugs (ethanol included) with postmortem examinations confirming diagnosis of heroin-related deaths were selected for this study. All cases presented high antemortem serum morphine values (a morphine concentration before death over 17ng/mL).

An experimental model and immunohistochemistry were used to investigate postmortem anti-heroin antibody expression in heroin-related deaths. The chosen analytes were morphine (free), morphine (total), and 6-monoacetylmorphine. Macroscopic and microscopic pathological findings in cases of heroin-related deaths are non-specific and may be inadequate to render a definitive diagnosis for forensic purposes. It is therefore evident that heroin-antibody positivity in kidney tubules, in hepatocytes, and in liver ducts would represent a normal physiological finding related to the elimination of metabolites of the heroin from blood circulation, and, in fact, an indirect finding of the elevated heroin concentration.

Finally, these findings on lung samples showed that positivity in the cytoplasm of myelomonocytes and in the intra-alveolar spaces can be correlated with acute respiratory depression heroin-related deaths.

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### Heroin-Related Death, Immunohistochemistry, Postmortem Diagnosis



## K16 Patterns of Natural Cannabinoids in Hair

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**Learning Overview:** After attending this presentation, attendees will understand the patterns in the relative abundances of Cannabidiol (CBD), Cannabinol (CBN), Tetrahydrocannabinol (THC), Tetrahydrocannabinol (THCV), and carboxy-THC found in hair samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the types of patterns and the variance in the patterns seen in the results of a large population of samples containing detectable levels of cannabinoids

The cannabinoids THC, THCV, CBD, CBN, and (-)-11-nor-9-carboxy- $\Delta$ -9-Tetrahydrocannabinol (C-THC) were determined in over 4,700 workplace hair samples. Confirmation of C-THC was by a previously published Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS) method.<sup>1</sup> Confirmation of THC, THCV, CBN, and CBD was by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) on an AB SCIEX™ QTRAP 6500+ LC/MS/MS with binary Shimadzu® LC-30AD pumps and a Leap PALHTC-xt autosampler system with Dynamic Load and Wash (DLW) washing. DLW wash 1 was 0.1% formic acid in H<sub>2</sub>O. DLW wash 2 was CH<sub>3</sub>CN:isopropanol:acetone (60:30:10). Chromatographic separation was accomplished using a 3.0mm x 50mm Phenomenex® Kinetex® 1.7 $\mu$ m C18 100 Å 150mm x 2.1mm. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in methanol. The method employed a gradient elution with a variable flow rate over 4.1 minutes.

The range of measured THC levels in the samples was from <Limit Of Detection (LOD) (5pg/mg) to 47,808pg/mg hair (note that some of these measured values were higher than the 5,000pg/mg upper limit of the assay). There was a wide variability in the relationship between parent THC and the metabolite C-THC. This variation is exemplified by two sets of samples with contrasting results. Among 79 samples that contained no THC above LOD, the C-THC ranged from 0.034–10pg/mg in hair. On the other hand, another set of 23 samples containing 10,000–47,808pg/mg THC in hair contained 0.2–21.9pg/mg C-THC in hair, not so very different from the C-THC range in the other set with no THC. These highly varying amounts of C-THC relative to THC are supported by other smaller studies.<sup>2-4</sup>

Only 26% of the samples contained THCV detectable by the method. The average THCV content when present was 1.77% of THC (SD 1.62, Median 1.38).

In THC-positive samples, 23% of samples contained less than 1% CBD, another 29% contained from 1% to 5% CBD, and 37% contained from 5% to 50% CBD. The roughly 11% of samples containing CBD at 50% or higher of the THC content likely consumed CBD and THC products, or a very few may have used only CBD products that contained THC.

Causes for the variabilities of parent and metabolite seen in these studies may be due to plant composition, storage, mode of use (oral, smoking, vaping), body weight, body fat composition, liver enzyme profile, or hair hygiene procedures. Still, the presence of C-THC remains the only definitive indicator of ingestion.

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### Cannabinoids, Hair, LC/MS/MS

## K17 Analysis of Fentanyl Analogs and Common Drugs

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**Learning Overview:** This presentation will allow attendees to describe analytical challenges that currently face forensic toxicology sections while trying to stay current with the opioid epidemic and ever changing drugs of abuse. Further, attendees will understand the steps utilized to validate an Ultra Performance Liquid Chromatography/quadrupole Time-Of-Flight (UPLC/qTOF) method according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the benefits and utility of toxicological analyses utilizing high resolution mass spectrometry.

The route most forensic toxicology testing laboratories implement to detect various drugs of abuse and their metabolites in blood specimens commonly includes a screening method followed by a confirmatory method of positive screening results. Due to the necessity for a greater sensitivity and level of specificity in confirmatory testing methods, instrumentation composed of Gas or Liquid Chromatography (GC or LC) capabilities coupled with Mass Spectrometry analysis (GC/MS, LC/MS, or LC/MS/MS) are the most utilized in the field of toxicology.<sup>1</sup>

The wide detection range and heightened sensitivity of Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) makes it a worthy screening method. The increasing prevalence of fentanyl analogs since 2014 has led to the rapid scheduling of these substances and thus a need for the ability to test for the compounds in laboratories.<sup>2,3</sup> As recently noted, over two-thirds of overdose deaths recorded in the United States involved opioids, and locations in North America experienced sharp increases of fatalities due to carfentanil.<sup>4</sup> This information demonstrates the need for toxicology laboratories to stay current with drug trends. This presentation details studies to validate 11 fentanyl analogs as well as several other drugs and metabolites using LC/qTOF/MS.

Thirty-two analytes, including 11 fentanyl analogs, and various other commonly encountered drugs/metabolites were validated for analysis in antemortem and postmortem casework according to SWGTOX standards. Validation studies consisted of selectivity/specificity, analyte recovery, carryover, ionization suppression/enhancement, sensitivity, stability, and method comparison. During specificity studies, one of the fentanyl analogs, p-methyl acetyl fentanyl, could not be distinguished from fentanyl. All other analytes demonstrated acceptable selectivity and specificity. Sensitivity of the 11 fentanyl analogs was between 0.5–1.0ng/mL. All other analytes had sensitivities between 5–5,000ng/mL. Post-extraction stability was determined to be seven days. Ionization suppression was considered to be unacceptable; however, it did not impact limits of detection, as all analytes validated were able to be detected at relevant concentrations in multiple matrix samples. To demonstrate the method as fit for its intended use, ten samples, which had been previously analyzed by reference laboratories, were analyzed. Nine of the 32 analytes were detected. No false positives or negatives were identified. This method presents a screening technique for fentanyl analogs and commonly abused drugs in antemortem and postmortem whole blood using LC/qTOF with forensically relevant limits of detection.

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### LC-QTOF, Fentanyl Analogs, Validation

## K18 Transforming Toxicology in South Africa—Illustrating Proof of Concept Using Data From Routine Carboxyhemoglobin (COHb) Analysis

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge on the use of a Radiometer ABL825 FLEX analyzer for the analysis of COHb content in postmortem blood, as well as the relevance of COHb determination in postmortem investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into South Africa's unnatural death case profile and the associated challenges relating to forensic investigations in a developing country.

**Introduction:** Forensic toxicology has historically been an underdeveloped field in South Africa. This has resulted in several challenges in the medicolegal investigation of unnatural deaths such as: excessive backlogs, poor infrastructure, and lack of expertise. The Forensic Toxicology Unit (FTU) was established in Cape Town in 2016 to support and grow forensic toxicology services in South Africa. In July 2020, the FTU launched a routine service for the analysis of COHb, with the aim of providing an efficient and quality-driven toxicological service to support the death investigation process.

**Methods:** All case samples were received, accessioned, and analyzed in accordance with newly developed laboratory standard operating procedures. Postmortem blood samples were analyzed for COHb content using an ABL825 FLEX analyzer. The analytical method was fully validated according to international guidelines. Duplicate analyses were performed for each case, and the average was reported.

**Results:** A total of 98 cases were analyzed over a three-month period. The manner of death was considered to be an accident in 57.1% of cases, followed by homicide in 14.3%, and suicide in 4.1% of cases. Smoke inhalation and injuries due to burns were frequently listed as the cause of death in accidental cases, whereas blunt force trauma or multiple injuries were most frequently listed in homicide cases. Of the 98 cases, 14.3% were children or infants (<18 years). COHb concentrations were above the reporting limit of 5% in 69.1% of cases (mean COHb concentration = 25.3%), with the highest concentration reported being 69.4%. The average turnaround time for reporting of results from specimen receipt was 9.5 days.

**Conclusion:** Fire-related deaths are a common occurrence in South Africa, particularly in vulnerable populations, and reliable COHb results are imperative for accurate cause of death determination. The successful implementation of routine COHb analysis has allowed forensic medical practitioners to conclude their postmortem investigations in record timeframes, the benefits of which are far reaching, not only for stakeholders involved, but also the community it serves. The FTU strives to continue developing its service capacity in order to transform toxicology in South Africa from its historical state into a new era.

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### Carboxyhemoglobin, Toxicology, South Africa

## K19 A Four-Year Comparison (2016–2019) of Toxicology Results in Suicide-Related Deaths From the West Tennessee Regional Forensic Center in Memphis, Tennessee

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**Learning Overview:** The goal of this presentation is to review any trends in substance use and suicide in cases from the West Tennessee Regional Forensic Center between the years 2016 to 2019.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing recent trends in toxicology results in suicides as well as help as a starting point for health care providers to approach concerns for suicide in their patients based on their substance abuse history.

Suicide rates have been increasing over the past two decades.<sup>1</sup> While substance abuse has been identified as a potential warning sign for suicide by the Centers of Disease Control, in recent years, few studies have examined the correlation between suicides and forensic toxicology results in the United States.<sup>2,3</sup> The current study reviewed toxicology profiles from known suicide cases examined at the West Tennessee Regional Forensics Center in Memphis, TN, from 2016 to 2019. The purpose of this research was to examine if any trends exist between substance use and suicide, including any trends in cause of death.

Toxicology reports from known suicides from 2016 to 2019 were reviewed, and age, sex, race, and cause of death were recorded. Cases without toxicology analysis and incomplete cases were excluded. The major categories for cause of death were gunshot wounds, asphyxia-related deaths, drug intoxication, blunt force trauma, sharp force trauma, and drowning. Age-based categories were made in ten-year increments. Toxicology results positive for common substances and drugs of abuse, alcohol, prescription drugs, and over-the-counter drugs were considered in this study. A total of 512 cases fit the above criteria in the time course examined.

Analysis of the data revealed 69.3% of cases detected substances in toxicology analysis. Of the 512 cases with toxicology reports from 2016–2019, 390 cases were male, which matches the overall trend for sex in suicides in the United States to be more common in males.<sup>1</sup> The most common toxicology result was the detection of multiple substances (33.2%), with alcohol being the most common substance detected (29.9%). Negative toxicology (30.6%) and Tetrahydrocannabinol (THC) (20.5%) were the next most common toxicology results found. Negative toxicology was the most common result found for those aged 70 and older (53.4%) as well as the second most common result in those aged 29 and younger (22.3%). Toxicology for THC was the most common result in those under 19 years of age (30.9%) as well as the third most common among those aged 20–29 (17.3%). Alcohol was one of the highest toxicology results for ages 20–69 (21.4%). Self-inflicted gunshot wounds were the most common cause of death (325 out of 512 cases). In these cases, negative toxicology (35.7%) was the most commonly found with alcohol (30.1%) being the second most common result. Hangings (101 out of 512) more commonly had multiple substances (33.7%) on toxicology followed by negative toxicology analysis (29.7%). The most common results in positive toxicology studies were THC, benzodiazepines, opioids, amphetamines, and cocaine.

Interesting trends were also detected in that opioids increased from 2016 to 2019, while benzodiazepines decreased over the same period. Only a minority of cases were positive for prescription antidepressants and anti-psychotics (9.9%); however, this result may be skewed due to the type of toxicology panel performed. Overall, these results show that toxicology in suicides is a topic that needs more research as well as a possible starting point for health care providers to approach concerns for suicide or suicidal ideations in patients based on their substance abuse history.

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### Toxicology, Suicide, Substance Abuse

## K20 Hydroxycocaine Concentrations in Head and Body Hair as Determined by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will have viewed an investigation of hydroxycocaine concentrations, as determined by LC/MS/MS, in head and body hair samples after an extended aqueous wash.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by imparting knowledge regarding methods useful for determining hydroxycocaine concentrations in hair samples after an extended aqueous wash. Interpretation of hydroxycocaine concentrations regarding cocaine ingestion is presented.

**Statement of Hypothesis:** It was hypothesized that hydroxycocaine isomers could be detected in head and body hair samples by LC/MS/MS. As part of this investigation, concentrations of hydroxycocaine isomers, effects of hair color, and stability of the hydroxycocaine isomers in hair were examined.

**Statement of Methods:** Head and body hair samples containing cocaine were also analyzed for Benzoylcegonine (BE), Cocaethylene (CE), norcocaine (Ncoc), para-Hydroxycocaine (p-OHCoc), meta-Hydroxycocaine (m-OHCoc), and ortho-Hydroxycocaine (o-OHCoc) by LC/MS/MS. The confirmation process consisted of a hair aliquot that was first washed using an extended aqueous method, followed by solid phase extraction and quantitation using either an AB SCIEX™ API 3200 LC/MS/MS for cocaine, BE, CE, and NCoc or an AB SCIEX™ 6500+ for p-OHCoc, m-OHCoc, and o-OHCoc. The confirmation used primary and secondary ions for each analyte in positive Multiple Reaction Mode (MRM). The cocaine LC/MS/MS method was linear from 25 to 15,000pg/mg hair for cocaine, BE, CE, and NCoc; the hydroxycocaine LC/MS/MS method was linear from 0.4 to 300pg/mg for o-, m-, and p-OHCoc.

**Results:** A total of 2,902 head and body hair samples were analyzed in this study. The percentage of samples having hydroxycocaine isomers in head hair below the Lower **Limit Of Quantification** (LLOQ) for p-OHCoc, m-OHCoc, and o-OHCoc was 4%, 2%, and 9.6%, respectively, and in body hair was 9%, 9%, and 4.3%. In this sample population, the median concentrations of p-OHCoc, m-OHCoc, and o-OHCoc were 1.6, 2.1, and 3.6 times greater, respectively, in body hair than head hair. The average concentrations of p-OHCoc, m-OHCoc, and o-OHCoc, respectively, as a percentage of cocaine in head hair were 1.143, 1.079, and 0.108%, and 0.788, 0.943, and 0.193% in body hair. The lower concentration of p-OHCoc and m-OHCoc as a percentage of cocaine in head hair versus body hair was significant (Z-test), with the higher concentration of o-OHCoc as a percentage of cocaine in body hair also significant (Z-test). With head hair, 5.0% of samples failed to be  $\geq 0.05\%$  of cocaine for one or both of m-OHCoc or p-OHCoc. In body hair, 10% of samples failed to equal  $\geq 0.05\%$  cocaine for one or both isomers. Comparison of % cocaine for black and brown head hair found a higher percentage of p-OHCoc in brown hair (Z-test), with no difference in % cocaine observed for p- and m-OHCoc in body hair (Z-test). Concentration ratios of m-OHCoc/o-OHCoc, and p-OHCoc/o-OHCoc were examined, with a ratio of 2 required for a head hair sample to be positive for cocaine ingestion, and body hair evaluated at ratios of 1.5 and 2. The effects of CE on hydroxycocaine concentrations were examined, with higher concentrations of p-OHCoc and m-OHCoc apparent in concentrations up to 3,000pg/mg CE in hair. Samples were analyzed after one year in storage at ambient temperature, with hydroxycocaine isomers proving stable over this time. Treatment of cocaine-positive samples with peroxide showed a large increase in o-OHCoc as percentage of cocaine as compared with smaller changes in p-OHCoc and m-OHCoc before sample washing. After an extended wash, only o-OHCoc showed an increase as percentage of cocaine.

**Conclusion:** Hydroxycocaines were analyzed for concentrations in hair, their concentrations in hair relative to cocaine and cocaethylene, and hair color effects. The hydroxycocaines were stable over one year storage at ambient temperature, with p-OH and m-OHCoc also stable to hair peroxide treatment. The p- and m-OHCoc isomers represent useful metabolites that can be used as biomarkers for evidence of cocaine ingestion.

### Hair, Hydroxycocaine, LC/MS/MS

## K21 Unusual Routes for Substance Abuse: Fatal Inhalation of a Fentanyl Patch

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**Learning Overview:** After attending this presentation, attendees will have the opportunity to appreciate the characteristics of intoxication that occurred following an unusual use of a fentanyl patch.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a unique case of a fatal inhalation of a fentanyl patch investigated by autopsy and toxicological analysis.

Fentanyl usually has been used by transdermal patches to treat cancer and non-cancer-related chronic pain. However, inappropriate or illegal application may cause fatal poisoning. Presented here is the case of a South American man, 29 years old, who was found dead on the road.

Fentanyl is a synthetic Mu-opioid receptor agonist, approximately 100 times more potent than morphine per dose. It has been widely used for anesthesia and to manage intense chronic pain. Fentanyl has been available for human application, both in the oral transmucosal and in the parenteral (such as transdermal) form. Recently, fentanyl transdermal patches with a matrix design (drug-in-adhesive) became more commonly used than those with a reservoir design. In Italy, fentanyl transdermal matrix patches are prescribed to alleviate cancer or non-cancer-related chronic pain, which is not alleviated by other analgesics, or when the oral administration route cannot be used.

A 29-year-old man was found dead on the street after attending a party with friends in which recreational drug use had taken place. An autopsy was performed approximately 48h after his death. On external examination, his body was 165cm in height and 80.0kg in weight. The body mass index was 29.4kg/m<sup>2</sup>. Postmortem lividity was highly expressed on his back. On the face and clothes were clear residues of vomit (not observed on the scene). There was minimal blood staining in the oral cavity. Internal examination revealed a dusky red color of the blood and marked multi-visceral congestion. At the opening of the respiratory tract, the protective film of a fentanyl transdermal patch was found inside the right main bronchus. The stomach contained 50mL of undifferentiated food material. No injuries were observed that could be directly related to death. Organ examination revealed non-specific signs of asphyxia; no other pathological findings likely to cause death in the remaining organs were observed.

The femoral blood alcohol concentration determined by Headspace/Gas Chromatography (HS/GC) was 0.22g/L. The immunochemical drug screening kit, Multiline Drug Test<sup>®</sup> for urine samples, was positive for cocaine, morphine, methadone, cannabinoids (THC), and benzodiazepines. A general screen for non-volatile organic compounds was performed using the GC/MS and the results revealed the presence of methadone, 2-Ethylidine-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP), morphine, 6-Monoacetylmorphine (6-MAM), cocaine, Benzoylcegonine (BEG), Ecgonine Methyl-Ester (EME), fentanyl, and alprazolam.

The postmortem signs and evidence gathered made it possible to identify the cause of death in a fentanyl intoxication following the unusual intake of the drug through chewing a patch. The state of intoxication induced a central depression underlying the respiratory failure. On the basis of the findings highlighted, it was also possible to demonstrate that respiratory failure was favored by the accidental inhalation of the protective film that caused a partial mechanical obstruction of the right main bronchus.

Cases of fatal intoxication from misuse—intentional or accidental—of therapeutic formulations of fentanyl have been described throughout the world. However, per research, there were no autopsy reports in Italy of intoxication caused by fentanyl transdermal patches abnormally consumed as in this case. This case represents further evidence of the social relevance and mortality related to the improper use of opioids intended for pain therapy.

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### Fatal Intoxication, Fentanyl Abuse, Patch Inhalation

## K22 Trends in Drug-Facilitated Crime and Sexual Assault in San Francisco, California

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**Learning Overview:** After attending this presentation, attendees will understand popularity trends in substances used in Drug-Facilitated Crime (DFC) and sexual assault in the San Francisco area.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness among forensic scientists of the trends in drug usage in drug-facilitated crime, including sexual assaults, in San Francisco from July 2015 to December 2018.

Drug-Facilitated Sexual Assault (DFSA) is a term used to describe a DFC where the sexual assault has occurred while the survivor was under the influence of a substance that incapacitated them to better control their faculties and engage them in non-consensual sexual activity. Drugs, including alcohol, compromise an individual's ability to consent to a sexual act by causing a diminished capacity. A perpetrator may take advantage of voluntary use of drugs or alcohol; alternatively, they may force or administer drugs or alcohol to the survivor unknowingly. Ethanol and marijuana are the two drugs most commonly detected in reported DFSA casework around the world; however, other drugs of interest include recreational drugs and hypnotic, sleep aid, and anxiolytic drugs.

The aim of this research was to determine if there were drug and demographic trends in reported DFSA cases in the City and County of San Francisco from July 2015-December 2018. Age, gender, time of incident and specimen collection, symptomology, voluntary and suspected drug use, and detected drugs in blood and urine data were obtained from the Office of the Chief Medical Examiner in San Francisco, who performs all DFSA forensic toxicological investigations within the city.

A total of 883 cases were examined; 745 identified as female, 116 identified as male, and 22 were not indicated. The median age of females was 26 and males was 29.5 years of age. The median elapsed time between the incident and specimen collection for the years 2015–2018 was 18.2 hours, with 13.5 hours, 20.6 hours, 19.3 hours, and 15.8 hours for 2015, 2016, 2017, and 2018, respectively.

The five most frequently occurring analytes in blood were cannabis and its metabolites (127), ethanol (118), methamphetamine (103), amphetamine (99), and cocaine and its metabolites (47). Urine analysis data showed several of the same substances within the five most frequent observations with ethanol (224), methamphetamine and amphetamine (200), cannabis and its metabolites (158), cocaine and its metabolites (142), and  $\gamma$ -hydroxybutyric acid (47) detections. In total, 448 analytes were detected through blood analysis and 669 analytes were detected through urine analysis.

Urine analysis data was used to determine trends in substances and gender. Ethanol was determined to be the most frequent drug used in DFSA within the female population, while methamphetamine was most commonly encountered in DFSA with males. Many substances used in DFSA can leave the body within 6 to 48 hours. As such, efforts to decrease the time between the incident and collection, as observed here, are beneficial.

Loss of consciousness, impaired memory, and drowsiness were the most common symptoms reported in these DFSA investigations. In cases involving loss of consciousness, the most common drugs detected were ethanol (156), marijuana (47), methamphetamine (31), and cocaine (12). Drugs associated with the loss of memory included the same drugs: ethanol (206), marijuana (62), methamphetamine (31), and cocaine (16). In investigations where drowsiness was reported, the most commonly detected drugs were also ethanol (121), marijuana (40), methamphetamine (21), and cocaine (12). It is notable that the Central Nervous System (CNS) stimulants methamphetamine and cocaine rank highly regarding reported sedative effects, although both drugs can be reported in this way as their concentrations decrease. In addition, it is known that there is some degree of overreporting of these effects based on victim symptomology.

In summary, demographic data from reported DFSA cases in San Francisco over a 3.5-year period indicate similar trends regarding age and sex to other DFSA reports. Unsurprisingly, alcohol and cannabis account for the highest number of drugs detected. Methamphetamine is very prevalent in DFSA cases in this region, particularly within the male population. Tracking drug use in DFSA casework can provide valuable information for the forensic science community, sexual assault survivor advocates, and controlled substance legislatures.

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### Drug-Facilitated Crime, Drug-Facilitated Sexual Assault, Trends

## K23 Postmortem Toxicology Trends in the United States Before and During the COVID-19 Pandemic

*Stephanie Marco, PhD\*, NMS Labs, Horsham, PA 19044*

**Learning Overview:** The goal of this presentation is to highlight the postmortem drug of abuse trends in United States cases received in 2019 and 2020.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new information on drug class and specific drug trends in postmortem toxicology before and during the COVID-19 pandemic.

**Introduction:** Since the beginning of 2020, the United States has experienced an unprecedented public health crisis due to the novel coronavirus (COVID-19). Measures implemented to reduce the spread of COVID-19 in the United States have significantly altered people's lifestyles. To investigate the effects of COVID-19 on postmortem drug trends, all United States cases submitted to NMS Labs during 2019 and 2020 were reviewed.

**Objective:** The objective of this study was to identify variations in the drug of abuse trends by drug class and the individual analyte in the postmortem cases that NMS Labs received during 2019 and 2020.

**Methods:** Drugs of abuse, specifically those belonging to the barbiturate, benzodiazepine, cannabinoid, hallucinogen, hypnotic/sedative, inhalant, opioid, and stimulant drug classes were identified in postmortem cases throughout the United States, including the District of Columbia, Puerto Rico, and the Virgin Islands. Cases that confirmed for any drug class between January 1 and July 31, 2019, and between January 1 and July 31, 2020, were included in this study.

**Results:** Between January 1 and July 31, 2019, 51,880 postmortem cases matching the criteria discussed in the methods section were identified. The prevalence of each drug class was determined as a percentage of total cases and are shown here in descending order: opioids (53%), stimulants (38%), cannabinoids (36%), benzodiazepines (26%), hypnotics/sedatives (1.0%), barbiturates (0.93%), hallucinogens (0.54%), and inhalants (0.49%). The most common analytes confirmed for each drug class were fentanyl (opioid, 28%), amphetamine (stimulant, 23%), delta-9 Tetrahydrocannabinol (delta-9 THC) (cannabinoid, 33%), alprazolam (benzodiazepines, 10%), ketamine (hypnotic/sedative, 1.0%), butalbital (barbiturate, 0.80%), phenacyclidine (hallucinogen, 0.43%), and 1,1-difluoroethane (inhalant, 0.49%).

A total of 40,496 cases between January 1 and July 31, 2020, were identified according to the previously described study criteria representing a decrease of 11,384 cases compared to the same period in 2019. This decrease may be due to several factors, including a reduction in overall travel and limited interpersonal contact. The prevalence of each drug class was similar to 2019, but with slight variations in the order of the least abundant drug classes: opioids (56%), stimulants (41%), cannabinoids (39%), benzodiazepines (23%), hypnotics/sedatives (0.95%), hallucinogens (0.78%), barbiturates (0.75%), and inhalants (0.18%). The most confirmed analytes for each class were identical to that of 2019 with slight variations in frequency: fentanyl (37%), amphetamine (23%), delta-9 THC (36%), alprazolam (9.0%), ketamine (0.90%), phenacyclidine (0.66%), butalbital (0.69%), and 1,1-difluoroethane (0.18%).

**Conclusions:** Modest differences in nationwide postmortem drug trends were observed in this study, suggesting that the effects of the COVID-19 pandemic were more nuanced. Opioid, stimulant, and cannabinoid confirmations increased by 3% from 2019 to 2020. By contrast, a decrease in hypnotic/sedative (0.05%), barbiturate (0.18%), inhalant (0.31%), and benzodiazepine (3%) confirmations was observed. The number of cases positive for each analyte varied slightly from 2019 to 2020, with the most noticeable being an increase in fentanyl (8.6%). These variations in drug trends may be due to several factors related to the COVID-19 pandemic—including reduced social interactions, disruptions in drug supply chains, and economic uncertainty—warranting further investigation.

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### Postmortem, Drug Trends, COVID-19



## K24 The Discovery of Gamma-Butyrolactone (GBL) in JUUL® E-Liquids

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**Learning Overview:** The goal of this presentation is to discuss the dangers of vaping. After attending, attendees should be able to recognize the significance of unlabeled components, including GBL, in e-liquids and their implications to the forensic community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of the presence of GBL in JUUL® products and its implications in forensic science.

**Background/Introduction:** GBL is a List I chemical because, while it has legitimate industrial uses, it can also be used to manufacture Gamma-Hydroxybutyrate (GHB), a controlled substance. Both are recreationally abused as club drugs and are used in drug-facilitated crimes, especially date-rape scenarios. GBL is a chemical with a variety of recognized industrial uses; it is a common solvent, flavoring agent, and is used in the manufacturing of other chemicals and pharmaceuticals. It is taken as a drug for a multitude of reasons, such as improving athletic performance, sexual performance/pleasure, and sleep and has also been used for relieving stress and depression, inducing relaxation, releasing growth hormone, and trimming body fat.

The presented study documents the discovery of GBL in e-liquid from “pod-mods” made by JUUL®. Though GBL has documented use as a flavorant, the discovery of GBL in e-liquids is alarming. There is a general lack of human clinical studies regarding the efficacy and safety of GBL ingestion. Another alarming consideration is the lack of any studies regarding inhalation of GBL and what effect this route of administration has on humans.

**Hypothesis:** The objective of this study was to evaluate the chemical composition of two e-liquids before use in a human clinical study designed to compare vaping an e-liquid with only nicotine to one that contains nicotine and Cannabidiol (CBD). The unexpected identification of GBL necessitated quantification.

**Methods:** JUUL® samples were provided by the Behavioral Health Research Lab at Virginia Commonwealth University and purchased from a number of local retail outlets as well as directly from JUUL® online. Samples were screened using Direct Analysis in Real Time-Mass Spectrometry (DART®-MS) followed by a 1:100 dilution in methanol analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). A liquid chromatography-triple quadrupole mass spectrometry method was employed for the quantification of GBL.

**Results:** DART®-MS and GC/MS analysis of the JUUL® pod e-liquid resulted in the identification of propylene glycol, vegetable glycerin, and nicotine along with benzoic acid and several compounds assumed to be flavoring agents: beta-damascenone, veratraldehyde, and triethyl citrate. Also identified were veratraldehyde propylene glycol acetal (a flavorant-propylene glycol adduct) and GBL. Evaluated samples were found to contain an average of 0.37mg/mL GBL.

**Conclusion/Discussion:** The compounds identified included carriers, nicotine, and flavorants, plus GBL. Quantification of the unlabeled GBL can potentially elucidate pharmacological and/or toxicological implications of inhaling these products. The presence of GBL in e-liquids at any concentration is a cause for concern. GBL is a Class I chemical. Also troublesome is the lack of studies regarding the effects of inhalation of GBL, which is a more direct route of administration. GBL is extremely potent, with reported doses ranging from 1–12mL when high-purity GBL is ingested orally. Finally, e-liquids often contain alcohol, with concentrations as high as 25%. GBL has been shown to have severe consequences when ingested with other drugs, including alcohol, due to drug interactions.

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### GBL, JUUL®, Mass Spectrometry

## K25 The Stability of Drug Analytes in Positive Umbilical Cord Tissue After Long-Term Frozen Storage

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**Learning Overview:** After attending this presentation, attendees will be able to evaluate the stability of 33 drug analytes in positive human umbilical cord samples after  $-20^{\circ}\text{C}$  sample storage for approximately one year.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting the proper specimen transportation and storage conditions for human umbilical cord tissue required to improve patient outcomes.

**Hypothesis:** Drugs of abuse remain detectable in umbilical cord tissue after one year of storage at  $-20^{\circ}\text{C}$ .

Drug abuse during pregnancy has risen rapidly, with the increase of opioid abuse over the past decade, boosting both the number of expectant mothers on opiate maintenance therapy and the incidence of Neonatal Abstinence Syndrome (NAS). As a result, evaluating *in utero* drug exposure during pregnancy is emerging as a critical service that hospitals should provide for both patient care teams and social services. Despite the long-time use of meconium in neonatal drug testing, many institutions are exploring alternative types of specimens, like umbilical cord tissue, because unlike meconium, umbilical cord tissue is available immediately after birth, is easy to collect under chain of custody, is available in sufficient quantity, and allows for a significant reduction in the turnaround time for results. However, the detection of drugs in umbilical cord tissue depends on many factors, including the extent of maternal drug use, the deposition of drug analytes in umbilical cord tissue, the analytical method, and, finally, the drug stability.

This research compared historical toxicology results with re-test confirmation results of the same umbilical cord tissue after frozen storage to evaluate stability of 33 drug analytes.

Sample preparation for drugs of abuse confirmation methods for cord tissue consisted of an external saline wash to remove contaminants followed by homogenization and extraction. The analytical method for cord tissue was performed on an AB SCIEX™ QTRAP 6500 triple quadrupole mass spectrometer equipped with an Electrospray Ionization source (ESI) for THCC and benzodiazepines and an AB SCIEX™ QTRAP 4500 triple quadrupole mass spectrometer equipped with an ESI for amphetamine, cocaine, and free opiates.

It was determined by the analysis of approximately 60 individual cord tissues that these 33 analytes remained stable in the cord tissue after  $-20^{\circ}\text{C}$  sample storage for approximately one year. The 33 analytes confirmed were 6-monoacetylmorphine; 7-amino clonazepam; alprazolam; amphetamine; benzoylecgonine; buprenorphine; butalbital; clonazepam; cocaine; codeine; diazepam; delta-9-Tetrahydrocannabinol (delta-9-THC); delta-9-carboxy THC; dihydrocodeine hydrocodol; 2-Ethylidene-1, 5-Dimethyl-3, 3-Diphenylpyrrolidine (EDDP); fentanyl; hydrocodone; hydromorphone; meperidine; methadone, methamphetamine, morphine, norbuprenorphine, nordiazepam, norfentanyl, norhydrocodone, normeperidine, noroxycodone, o-desmethyltramadol; oxycodone; oxymorphone; tramadol; and zolpidem.

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### Drug Stability, Umbilical Cord, Forensic Toxicology

## K26 The Evaluation of Extraction Parameters for the Analysis of Authentic Hair Reference Material (HRM) in Forensic Hair Testing Using Statistical Design of Experiments (DoE)

Brianna Spear\*, Miami, FL 33176; Anthony P. DeCaprio, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will understand the value of authentic HRM and DoE for evaluating best practice methods in forensic hair testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the most effective parameters for analysis of oxycodone, alprazolam, nordiazepam, and methamphetamine in hair, providing data toward creating standardized procedures for hair testing and understanding the benefits of authentic HRM.

**Hypothesis:** The hypothesis of this work is that DoE is a valuable approach to evaluating the most effective methods for extraction in forensic hair analysis.

There are many differing opinions regarding the optimal methods for hair analysis, especially regarding the pretreatment parameters. Extraction methods include enzymatic, acid/base, and solvent techniques, each with their own advantages and disadvantages. Other parameters include incubation times, temperatures, and size of the extracted hair particles. To assess pretreatment parameters for drugs of abuse, the statistical technique known as DoE is useful. DoE allows for evaluating both the individual roles and the combinatorial associations between multiple variables and drug extraction efficiency.

Previous research in the laboratory has focused on incorporated HRM, which is prepared in a laboratory at a specified drug concentration, but that does not necessarily reflect the mechanism by which drug incorporates into hair *in vivo*. In addition, using this approach, some drugs are not incorporated into hair at a high enough concentration for use as HRM. In contrast, authentic HRM, which is prepared by mixing hair from drug users to achieve specific drug concentrations, is an effective matrix for drug testing, since drug is incorporated into the hair through natural processes of the body.

Authentic HRM containing the drugs of interest was obtained from a commercial source. Samples of 20mg each were weighed into steel milling jars. The samples underwent extraction parameters determined using a 2<sup>3</sup> full factorial DoE matrix. Each sample was randomly assigned specific design points made up of combinations of factors of interest in extraction protocols. These included extraction solvent/sample size ratio (12.5 or 25µL/mg), particle size (pulverized into a powder using a Retsch® MM200 ball mill with chrome-steel milling beads at 3,800rpm for 30s or cut into snippets with scissors), and extraction time (2 or 24h). The samples were extracted using a solvent swelling technique in which the hair was incubated in methanol:acetonitrile:2mM ammonium formate (25:25:50) at 37°C. The samples were then transferred into Eppendorf tubes and centrifuged for 30min. Post-centrifugation, the eluent was subjected to solid phase extraction using a mixed mode C18 + cartridge. An Agilent® 1290/6460 Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) was used for analysis.

Analysis Of Variance (ANOVA) F-tests were performed post-analysis to determine if the parameters were significantly different. The ANOVA F-test indicated that high-level interactions, such as interactions between 2-3 factors (methamphetamine and oxycodone), as well as all interactions (alprazolam), were significant in the extraction of drugs of interest from the hair. This result suggests that studying variables individually and in combination with each other is important in the evaluation of forensic hair analysis methods. A consensus statement was made based on the design points with the highest percent recovery to determine which parameters were most effective for extraction of the drugs of interest. These included pulverizing the hair prior to a 24h extraction in 12.5µg/mg extraction solvent (oxycodone, nordiazepam, and alprazolam) and cutting the hair into snippets prior to a 2h extraction in 12.5µg/mg extraction solvent (methamphetamine). In conclusion, DoE is a valuable approach for determining effective extraction protocols for drugs of interest using authentic HRM.

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**Forensic Hair Analysis, Statistical Design of Experiments, Authentic Hair Reference Material**

## K27 Recommendations for Drug Testing in Driving Under the Influence of Drugs (DUID) and Motor Vehicle Fatality Cases—2021 Update

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**Learning Overview:** After attending this presentation, attendees can compare their cutoff limits to community consensus recommendations for Tier I compounds and evaluate implementing Tier II compound testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting updated recommendations for laboratory testing in DUID and traffic fatality investigations from the National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID) to improve standardization and data collection to better characterize the DUID problem.

The purpose of this project was to update the 2017 recommendations by re-evaluating Tier I and Tier II scope and cutoffs for screening and confirmation and potential changes to either scope. The recommendations were based on a survey sent to laboratories throughout the United States and Canada about testing practices, scope of testing and cutoffs, matrices tested, and compliance with the 2017 recommendations. Laboratories were also surveyed on drug prevalence and their laboratory methods and resources. The survey was sent via SurveyMonkey™ to laboratories confirming their participation in DUID testing, and ultimately 65 laboratories completed the survey.

A virtual consensus meeting, comprised of 23 forensic science practitioners who participated in the survey, was held to re-evaluate the 2017 recommendations following analysis of the survey results. Participants were selected to include a variety of perspectives based on laboratory type, the number of DUID and traffic fatality cases tested annually, matrices tested, and geographical location. As a result of the consensus meeting, no compounds were added or removed from Tier I. The screening and confirmation cutoffs for carisoprodol in blood and urine were raised to 1,000ng/mL. The blood confirmation cutoff for norbuprenorphine and the urine confirmation cutoff for fentanyl were raised to 1ng/mL. Trazodone and Difluoroethane (DFE) were added to Tier II due to their increased prevalence. The cutoffs for oral fluid were re-evaluated based on ongoing research, current levels of testing, and concentrations in DUID cases. For clarification, the Tier I cutoffs reflect free concentrations and hydrolysis is not required.

While gabapentin has shown an increase in prevalence, promotion to Tier I was difficult to justify at this time due to poly-drug use. Gabapentin is typically present together with other drugs, such as opioids and anti-depressants, at high concentrations. Despite relatively low prevalence for 3,4-Methylenedioxymethamphetamine (MDMA), 3,4-Methylenedioxyamphetamine (MDA), oxazepam, temazepam, oxymorphone, and hydromorphone, these compounds will remain in Tier I due to their relevance in assessing metabolic pathway for parent compounds and usefulness in determining drug abuse patterns. Similarly, when a laboratory is limited to screening by immunoassay, both 7-aminoclonazepam and clonazepam should be tested; however, if other screening technologies are available to the laboratory, then it is acceptable to screen for only 7-aminoclonazepam.

The consensus panel agreed that urine is an inferior matrix to blood and oral fluid and provides less information regarding potential impairment collaboration in DUID and motor vehicle fatality cases. This iteration of recommendations will be the last to include urine as a matrix for testing Tier I and Tier II compounds. Laboratories testing urine for DUID and motor vehicle fatality cases should consider testing blood and/or oral fluid to assess a driver's recent drug use. The panel has also updated recommendations for oral fluid testing requirements, based on increasing amounts of available data and increased attention to oral fluid as a matrix in these cases.

Updates to the 2017 cutoffs and recommended test menu will be distributed by NSC ADID in early 2021.

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### DUID, Cutoffs, Guidelines

## K28 Canadian Blood Drug Concentration Regulations and Drug-Impaired Driving Cases: A Snapshot of Findings in the Province of Québec

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**Learning Overview:** The goal of this presentation is to provide a portrait of findings (prevalence, blood concentrations) for the ten substances covered by the new Canadian Blood Drug Concentration Regulations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with an assessment of the impact of such a legislation, and observed blood concentration distributions, contributing to the discussion regarding legal thresholds for these substances.

**Introduction:** On June 26, 2018, a new Driving Under the Influence of Drugs (DUID) law came into force in Canada. Some key elements of this legislation were increasing ease of access to suspects' blood samples, and introduction of *per se* regulation for 10 drugs. This study assesses the impact of this legislation on casework for the province of Québec and draws a portrait of findings for these 10 substances targeted by the new regulation.

**Method:** Blood samples were systematically analyzed by liquid chromatography coupled to tandem mass spectrometry. For all DUID cases treated by the laboratory, data related to drivers (age, gender), the arrest (date, time, location, investigative tools), the sample(s) (collection date and time, type of biological matrix) and findings (detected analytes and concentrations) were compiled using a Microsoft® Excel® database. Summary statistics and data visualization were generated using Excel®, R, and RStudio®.

**Results:** Implementation of this new legislation led to a 226% increase in whole blood DUID cases. Between June 26, 2018, and August 1, 2020, 808 such cases were treated. Of these, 572 (71%) did test positive for at least one of the drugs covered by the Blood Drug Concentration Regulations, 535 (66%) having at least one drug over the *per se*. Prevalence and concentration data are summarized in the following table.

Drugs	<i>Per se</i>	Reporting limit	Prevalence	Median	Range
Tetrahydrocannabinol (THC)	2ng/mL* 5ng/mL	0.5ng/mL	38% (n=308)	3.7ng/mL	0.7–53
Methamphetamine	Any DL**	10ng/mL	37% (n=296)	210ng/mL	14–1,480
Cocaine	Any DL	10ng/mL	14% (n=114)	62ng/mL	14–1302
Gamma-hydroxybutyrate (GHB)	5mg/L	10mg/L	14% (n=110)	108mg/L	11–417
Ketamine	Any DL	10ng/mL	3% (n=22)	159ng/mL	15–988
Psilocin	Any DL	5ng/mL	0.1% (n=1)	Not quantified	
Psilocybin	Any DL	Not analyzed	NA	NA	NA
Lysergic acid diethylamide (LSD)	Any DL	1ng/mL	0.0% (n=0)	NA	NA
Phencyclidine (PCP)	Any DL	10ng/mL	0.0% (n=0)	NA	NA
6-Monoacetylmorphine	Any DL	5ng/mL	0.0% (n=0)	NA	NA

\*Summary offense (less severe): 2ng/mL, hybrid offense: 5ng/mL. \*\*Any detectable level.

Other prevalent illicit drug findings in whole blood samples included Methylenedioxymethamphetamine (MDMA, 33 cases), flubromazolam (26), flualprazolam (21), and etizolam (11). Of the MDMA cases, 9 did not show concomitant presence of a regulated drug, 8 synthetic benzodiazepines cases were in the same situation. Other prevalent prescription drug findings (which may also be used recreationally) included citalopram/escitalopram, diphenhydramine, clonazepam/metabolite, venlafaxine, pregabalin, and quetiapine.

**Conclusion:** Implementation of new *per se* regulations in Canada and easing up the access to blood collection did have a significant impact on caseload. The regulation was successful in targeting the most prevalent illicit drugs. MDMA and synthetic benzodiazepines, while not covered by this legislation, also show significant prevalence.

### DUID, *Per Se*, Blood Concentration

## K29 Pennsylvania Driving Under the Influence of Drugs (DUID) Trends: 2010–2020

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**Learning Overview:** After attending this presentation, attendees will better understand how DUID testing and trends have changed in Pennsylvania since 2010.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing drug trends in Pennsylvania's DUID population over the past ten years.

The drug market has transformed dramatically over the past ten years with the introduction of novel designer drugs and a surge in popularity for fentanyl, a potent opioid. As a result, the scope of DUID testing and drug positivity has also changed. The National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID) has published two updates to its Recommendations for Toxicological Investigations of Drug-Impaired Driving and Motor Vehicle Fatalities since 2010 to account for these changes. This work documents the changes in testing scope and drug positivity over the past ten years in Pennsylvania DUID blood cases.

**Method:** Antemortem blood specimens submitted for drug analysis, during DUID investigations in Pennsylvania, between January 2010 and July 2020 were reviewed. Cases were screened by Enzyme-Linked Immuno-Sorbent Assay (ELISA), alone or in combination with Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) beginning in 2014. Prior to 2014, Gas Chromatography/Mass Spectrometry (GC/MS) was used in place of the LC/TOF/MS. Submissions where some or all testing was not performed were excluded. Percent positivity for drug or drug classes was calculated based on the total number of routine DUID submissions received.

**Results:** The total number of DUID panels ordered by Pennsylvania clients increased approximately ten-fold from 1,593 in 2010 to 16,513 in 2019. Testing scopes for the routine ELISA screen have increased from an 8-panel test in 2010 to a 15-panel test in 2020. Drugs such as buprenorphine, tramadol, and fentanyl were added in January 2018 to align with the NSC ADID recommendations. Furthermore, the LC/TOF/MS panel is routinely updated to include an ever-growing library of novel psychoactive substances, including designer opioids and designer benzodiazepines. Cannabis was the most identified drug across all ten years with percent positivity approximately 50% or above each year. Drugs such as fentanyl, methamphetamine/amphetamine, mitragynine, and xylazine all experienced increases in positivity during the evaluation period, while opiates, oxycodone, and prescription benzodiazepines experienced steady positivity declines. The most prominent increases occurred in the fentanyl and methamphetamine assays where percent positivity increased approximately 14% for both drug classes. Positivity increases continue to be observed into 2020 for these two compounds. Between 2019 and the first half of 2020, fentanyl positivity increased approximately 3%, while the methamphetamine positivity increased 5%. Additionally, opiate and oxycodone assays have recently experienced decreases in positivity after each achieving maximum positivity in 2015 of 16% and 8%, respectively. Percent positivity of 6% for opiates and 3% for oxycodone were observed during the first half of 2020. Recent decreases in benzodiazepine positivity have been observed with the lowest positivity rates in ten years occurring between 2018 and 2020. This coincided with an increased positivity for designer benzodiazepines, such as etizolam and clonazepam.

**Conclusion:** DUID testing has changed dramatically over the past ten years. Testing scopes have expanded to include new and prevalent drugs capable of causing impairment. At the same time, submissions increased ten-fold. The popularity of drugs such as fentanyl, methamphetamine, and designer benzodiazepines has increased while others such as opiates, oxycodone, and prescription benzodiazepines have seen recent decreases. In order to best serve the forensic science community, DUID testing laboratories should adapt to changing drug trends.

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### DUID, Driving, Trends

## K30 The Impact of Reducing the Ethanol Threshold for Performing Drug Testing in Driving While Intoxicated (DWI) Cases

Teresa R. Gray, PhD\*, Houston, TX 77054

**Learning Overview:** Forensic toxicology laboratories often employ tiered testing protocols for Driving Under the Influence (DUI)/DWI cases where all cases are tested for Blood Alcohol Concentration (BAC), but drug testing is performed only if the BAC is below a laboratory-defined threshold. The BAC threshold for drug testing varies by laboratory but is usually between 0.08–0.15g/100mL. For many years, the Harris County Institute of Forensic Sciences used a drug testing threshold of 0.17g ethanol/100mL. The decision point was lowered to 0.10g ethanol/100mL in June 2015 in response to recommendations from county policy makers. After attending this presentation, attendees will understand how the lowered decision point impacted drug positivity rates and case throughput.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing attendees to assess whether the change in drug testing policy was effective and would have significantly impacted impairment interpretation for cases with BACs between 0.10–0.17g/100mL.

**Methods:** DUI/DWI cases were tested for volatiles using dual column headspace gas chromatography-flame ionization detection. Drug screening, if required, was performed with a 9- or 11-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) and/or liquid chromatography/time-of-flight mass spectrometry targeting at least 34 drugs or metabolites. Confirmation testing was performed with gas or liquid chromatography coupled with single or tandem mass spectrometry. DUI/DWI case results from 2.5 years before and after the threshold change (January 2013–December 2017) were extracted from the laboratory's information management system.

The potential for drug impairment (i.e., pharmacological action, ethanol-drug interaction, drug concentration) was assessed for cases with BACs between 0.10–0.17g/100 mL, understanding that predicting the likelihood or magnitude of impairment based on drug presence or concentration alone is fraught with difficulty. Cases were divided into five groups based on drug findings: (1) negative drug results; (2) non-impairing drugs or inactive metabolites; (3) only Central Nervous System (CNS) depressants; (4) only cannabis, defined as the presence of  $\Delta$ -9-Tetrahydrocannabinol (THC) above the detection limit of 1ng/mL; and (5) CNS stimulants or multiple drug classes.

**Results:** With a 0.17g/100mL BAC threshold, approximately 50% of the 5,059 DWI cases from January 2013–June 2015 were drug tested; by reducing the threshold to 0.10g/100mL, less than 25% of the 7,503 DWI cases from July 2015–December 2017 were drug tested, saving the laboratory over 2,000 drug screens and hundreds of confirmation tests. Changes to staffing levels, testing procedures, and casework priorities precluded meaningful analysis of how the threshold change impacted turnaround time.

The drugs found in casework were not impacted by the reduced threshold, as the ten most prevalent analytes remained consistent: norcarboxy-THC, THC, alprazolam, benzoylcegonine, hydrocodone, meprobamate, acetaminophen, carisoprodol, cocaine, and cocaethylene. Heroin (measured as 6-acetylmorphine), methamphetamine/amphetamine, and phencyclidine use in drivers appears to be more commonly associated with low (<0.10g/100mL) or no alcohol use, as positivity rates were greater after the reduction in drug testing threshold.

Among drivers whose BAC were between 0.10–0.17g/100mL, most (~65%) had negative drug tests and another ~6% were positive for only non-impairing drugs or metabolites. Approximately 6% were positive for one or more CNS depressants, usually at therapeutic concentrations. For these CNS depressant cases, the drugs would, at most, enhance the ethanol effect. Approximately 10% were positive for cannabis only. Concurrent alcohol and cannabis use have been associated with greater impairment than alcohol alone; therefore, the presence of THC was considered as having “significant” impacts on interpretation. Finally, approximately 12% of drivers had CNS stimulants or multiple drug classes. For this group, the impact of the drug(s) on intoxication interpretation could vary depending on the type of drug, individual tolerance, the phase of intoxication (e.g., the rush or crash of stimulants), and concentration. To be conservative, the presence of CNS stimulants or multiple drug classes was considered as having “significant” impacts on interpretation.

Since the implementation of the new testing protocol, stakeholders have provided little feedback. Additional testing is rarely requested and is usually limited to higher level offenses (e.g., intoxication manslaughter or felony murder).

**Conclusions:** Changing the drug testing threshold from 0.17 to 0.10g/100mL dramatically reduced the number of cases requiring drug testing without significantly impacting impairment interpretation. The drug results in over 70% of drivers with BACs between 0.10–0.17 were negative or non-impairing. While the change in testing policy was effective in Harris County, these results may not be applicable to other jurisdictions due to differences in alcohol-impaired driving prevalence, drug usage rates, laboratory testing capability and staffing resources, law enforcement programs targeting drugged driving, and local statutes regarding alcohol- and/or drug-impaired driving.

### DWI, Drugged Driving, Ethanol

## K31 The Rise of Polypharmacy-Impaired Driving Involving Fentanyl

*Nicholas B. Tiscione, MS\*, Palm Beach County Sheriff's Office, West Palm Beach, FL 33406*

**Learning Overview:** After attending this presentation, attendees will have increased their competence in the interpretation of and need for fentanyl testing in impaired driving investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining the increasing incidence, polypharmacy, and a case report of fentanyl in impaired driving investigations.

**Introduction:** A large amount of research has been presented on the impact of the opioid epidemic, and in particular fentanyl, on drug overdoses in death investigations. Conversely, relatively few reports have described the effect of increasing fentanyl use on impaired driving investigations. Herein data from a laboratory in the southeastern United States is presented.

**Methods:** For all Driving Under the Influence of Drugs (DUID) cases, a volatile analysis was performed by headspace Gas Chromatography (GC) with simultaneous flame ionization and Mass Spectrometry (MS) detection. Screening for other drugs was conducted using a basic extraction with scan GC/MS and an 11-panel Enzyme-Linked Immunosorbent Assay (ELISA) for amphetamines, barbiturates, benzodiazepines, buprenorphine, carisoprodol, cocaine/benzoylecgonine, fentanyl, methamphetamines, opiates, oxycodone/oxymorphone, and cannabinoids. All positive results were confirmed with GC/MS and/or liquid chromatography with tandem mass spectrometry. The ELISA cutoff for fentanyl was 1.0ng/mL prior to 2018 and lowered to 0.5ng/mL in 2018. The limit of quantification for fentanyl was 1.0ng/mL

**Results:** From 2014 to 2019, 158 out of a total of 580 (27%) drug-positive blood specimens collected for impaired driving investigations were positive for fentanyl. Six cases (4%) were also positive for midazolam, indicating that fentanyl may have been administered post-driving incident by Emergency Medical Services (EMS) in a small number of cases. Fentanyl concentrations ranged from 1.0–25ng/mL. A substantial increase in fentanyl cases was observed in 2016 compared to a positivity rate of 7% in 2014 and 10% in 2015. From 2016 to 2019, greater than 30% of the total drug-positive blood specimens contained fentanyl, with 45% in 2018 and 40% in 2019. In drug-impaired driving investigations fentanyl was the most frequently identified drug in 2018 and second most frequently identified drug in 2019. This trend continued into 2020. In the first six months of 2020, fentanyl was the most frequently identified drug; identified in 41% of blood positive impaired driving cases.

Other drugs were identified in the vast majority of the fentanyl cases. From 2014 to 2019, 95% of the fentanyl-positive cases also contained another drug. The most common combinations of other active drugs were other opioids (66%), benzodiazepines (46%), stimulants (30%), Tetrahydrocannabinol (THC) (28%), and ethanol (11%).

**Case Report:** An officer witnessed a vehicle strike a bicycle. The vehicle swerved into another vehicle well after hitting the bicycle in what the officer described as a delayed action to avoid hitting the cyclist. The driver did not stop after the crashes and had to be pursued. After stopping, the driver was observed to be aggressive and combative. He had extremely constricted pupils, was on-the-nod, and passed out just prior to the second of two blood draws ~5 hours after the crash. The driver was under close observation after the crash and was taken to the hospital after the second blood draw. Naloxone was not administered at the scene. The driver had a recent history of overdose incidents which required responses from EMS. The same paramedics that responded to the crash scene were also called later that day for an overdose at the driver's home after he was released from the hospital.

Whole blood specimens were collected at two different times into evacuated glass gray-stoppered vials containing sodium fluoride and potassium oxalate; ~1 hour after the crash and ~5 hours after the crash. No volatiles were detected in the blood specimen from the first draw. The blood drug analysis identified cocaine at 47ng/mL, Benzoylecgonine (BZE) at 309ng/mL, fentanyl at 35ng/mL, oxycodone <10ng/mL, THC at 3.3ng/mL, hydroxy-THC at 2.5ng/mL, and carboxy-THC at 77ng/mL. No volatiles were detected in the blood specimen from the second draw. The blood drug analysis identified cocaine at 9.4ng/mL, BZE at 277ng/mL, fentanyl at 39ng/mL, THC at 2.8ng/mL, hydroxy-THC at 1.8ng/mL, and carboxy-THC at 54ng/mL.

**Discussion/Conclusion:** The incidence of fentanyl in impaired driving cases has increased dramatically since 2014. From 2018 to the first six months of 2020, at least 40% of drug-positive blood DUID cases each year have contained fentanyl. Other drugs were identified in the vast majority (95%) of the cases in this jurisdiction. With the potential for significant tolerance, as demonstrated in the presented case report, observed effects may be unexpected. Individuals may maintain consciousness even at levels expected to produce severe respiratory depression and death. Fentanyl impairment can be significant and poses a risk for traffic safety.

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### Fentanyl, DUID, Polypharmacy



## K32 Houston Cocktail: Cases of Driving Under the Influence of Hydrocodone, Alprazolam, and Carisoprodol

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**Learning Overview:** After attending this presentation, attendees will have learned about the prevalence, blood toxicological profile, and demographic distributions of apprehended drivers in Houston, TX, who tested positive concurrently for hydrocodone, alprazolam, and carisoprodol—a dangerous combination known as the “Houston Cocktail” or “Holy Trinity.”

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing important regional information on the toxicological and demographic patterns of impaired driving cases in Houston that involved the specific combination of the opioid, benzodiazepine, and muscle relaxant. The long-term objective is to raise awareness and aid in implementing enforcement and prevention programs of the impaired driving cases involving the Houston Cocktail.

Concurrent use of opioids, benzodiazepines, and skeletal muscle relaxants potentiates the “high” drug effect and respiratory depression via interactions of  $\mu$ -opioid and **Gamma-Aminobutyric Acid** (GABA<sub>A</sub>) receptors.<sup>1</sup> In the early 2000s when abuse of prescription drugs began to spike, a potent combination including hydrocodone, alprazolam, and carisoprodol emerged that may give users heroin-like euphoria. *Houston Chronicle* reported more than 144,000 prescriptions for the three drugs in combination, while medically unwarranted, were dispensed in 2009 and almost 70% came from Harris County, where Houston is located.<sup>2</sup>

This research evaluated Driving While Intoxicated (DWI) or Driving Under the Influence of Drugs (DUID) cases that tested positive for hydrocodone, alprazolam, and carisoprodol, between 2015 and 2019; no cases in 2014 were tested positive for all three drugs. The blood samples were collected from drivers and submitted by the Houston Police Department (HPD). They were subsequently analyzed for alcohol and drugs by reference laboratories or the Houston Forensic Science Center (HFSC). Toxicological findings and demographic information, including age, sex, and race, were evaluated for the impaired driving cases, which tested positive for hydrocodone, alprazolam, and carisoprodol simultaneously in blood. The drugs were analyzed by gas chromatography/mass spectrometry or liquid chromatography/tandem mass spectrometry. The limits of detection were 5–20ng/mL for alprazolam and hydrocodone and 0.2 or 0.5 $\mu$ g/mL for carisoprodol, depending on the laboratories; carisoprodol and meprobamate in 12 cases analyzed by HFSC were reported qualitatively with the cutoff of 0.5 $\mu$ g/mL.

A total of 80 DWI/DUID cases positive for hydrocodone, alprazolam, and carisoprodol in blood were identified in which the traffic offense occurred between May 2015 and December 2019 (no positive cases in January–April 2015). The number of the Houston Cocktail cases increased from 9 in 2015 to 22 in 2019. However, because the total number of DWI/DUID cases also increased over the years, the proportion of the Houston Cocktail cases in the drug-positive DWI/DUID cases decreased from 3% in 2015 to 2% in 2019. Among the 80 Houston Cocktail cases, the mean (median, range) concentrations were 75 (61, 6.9–322) ng/mL for hydrocodone, 58 (48, 5.8–180) ng/mL for alprazolam, and 3.9 (3.0, 0.3–14;  $n=68$ )  $\mu$ g/mL for carisoprodol; 80 (100%) and 23 (29%) cases were also positive for meprobamate (mean 13; range 1.2–41 $\mu$ g/mL) and hydromorphone (1.8; 1.0–3.3ng/mL), respectively. Forty-six percent of those cases were female and 54% were male; 44% were Black, 46% were White, and 10% were other races as identified by the arresting officer. Mean (median) age of the drivers was 36 (34) years, ranged from 22 to 60 years. The majority (55%) were between 31–40 years of age; 23% between 22–30 years of age, 14% in the 41–50 years of age range, and 9% between 51–60 years of age. Twenty-three percent of the cases were positive for the Houston Cocktail only; 21% had one other drug/metabolite, 28% two, 18% had three, and 11% had four or more additional drugs/metabolites. Of the 80 cases, cannabinoids were the most frequently detected analytes (35%), followed by codeine (11%).

Between 2000 and 2009, the Texas Poison Center received 1,295 ingestion cases of the hydrocodone-alprazolam-carisoprodol combination, 34% of which came from Harris County.<sup>3</sup> The common adverse effects included drowsiness, slurred speech, tachycardia, confusion, respiratory depression, and coma.<sup>3</sup> The Houston Cocktail-positive drivers were distinguished from the general drug-positive drivers by a larger representation of females and younger users. The present study showed that despite a significant health risk, drivers in Houston continue to use this deadly drug combination. The risk is further exacerbated by the fact that most drivers had yet other drugs in their systems besides the three drugs.

### Reference(s):

1. Horsfall and Sprague. The pharmacology and toxicology of the “Holy Trinity.” *Basic Clin Pharmacol Toxicol.* 2017;120:115-119.
2. Olsen. Dangers of prescription drug abuse hit home. *Houston Chronicle.* July 18. 2010. <https://www.chron.com/news/houston-texas/article/Dangers-of-prescription-drug-abuse-hit-home-1607219.php>.
3. Forrester. Ingestions of hydrocodone, carisoprodol, and alprazolam in combination reported to Texas Poison Centers. *J Addict Dis.* 2011;30:110-115.

### Driving While Intoxicated, Houston Cocktail, Holy Trinity

## K33 An Evaluation of Drug-Facilitated Sexual Assault Cases in the City of Houston, Texas, From 2014 to 2019

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**Learning Overview:** After attending this presentation, attendees will have learned about the prevalence, urine and blood toxicological profiles, and demographic distributions of Drug-Facilitated Sexual Assault (DFSA) cases in Houston, TX.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the drug trends and demographics involved in suspected sexual assaults in Houston, TX, over a period of five years.

DFSA are an important public health and safety concern. In 2019, 18,057 sexual assault incidents were reported in Texas, with 8% reported to the Houston Police Department.<sup>1</sup> The true prevalence of DFSA is difficult to determine as many go unreported, there may be a delay in sample collection, or the scope of forensic toxicology laboratories may be limited. Understanding the trends in drugs involved in DFSA cases can assist agencies and laboratories in the investigation and toxicological analysis for cases of suspected DFSA.

This study examined urine and blood toxicology test results and case information associated with sexual assault cases submitted to the Houston Forensic Science Center (HFSC) with offense dates between January 1, 2014, and December 31, 2019. Total numbers of requests received, sexual assault cases analyzed, and demographics were examined. The prevalence of drugs found in confirmed positive cases and the associated demographics were also examined. Blood, if available, was analyzed for ethanol by dual column headspace gas chromatography with flame ionization detection. Drug screen analysis was performed preferably for urine samples using Enzyme-Linked Immunosorbent Assay (ELISA). Due to limited resources, after screen reports are issued, the laboratory only confirms positive screen results upon request. Drug confirmation analysis was performed using gas/liquid chromatography/mass spectrometry either in-house or by external laboratories. Demographic data, including age, sex, and race, were evaluated.

Blood and urine samples were analyzed for alcohol and drugs from 997 cases related to sexual assault, accounting for 16% of toxicology analysis requests received by HFSC. The total number of cases with blood only was 51 cases, 113 with urine only, and 840 with both (1,004 samples total).

Analysis of the demographic data showed alleged victims were 73% female, 4.9% male, and 23% unknown. Race demographics showed alleged victims were 24% White, 14% Black, 5.4% Hispanic, 0.6% Asian, 9.2% Other, and 47% Unknown. The average age was 26 ( $n=1,004$ ; 2.7% unknown), with the range from 4 to 78 years old.

An average of 71% (712 samples) were presumptive positive for at least one drug or drug class. Ethanol was the most prevalent drug detected in 171 samples with an average blood alcohol concentration of 0.097 (range 0.011–0.336) g/100 mL, and in 148 samples ethanol was the only drug determined to be present (average 0.098, 0.011–0.336) g/100mL. A total of 129 samples (116 urine, 13 blood) were confirmed positive for drugs other than ethanol only. There were 23 samples that were confirmed for both ethanol and other drugs. The most prevalent analyte other than ethanol was 11-Nor-9-Carboxy-Delta-9-Tetrahydrocannabinol (THC-COOH) being present in 32% of confirmed samples. The next most prevalent analytes were benzoylecgonine (14%), alprazolam (11%), and amphetamine (11%). All other analytes confirmed positive in less than 10% of samples. Of all confirmed positive samples, victims were 78% female, 4.0% male and 18% unknown. Race demographics showed victims were 28% White, 12% Black, 5.3% Hispanic, 6.0% Other, 0.3% Asian, and 48% Unknown. The average age was 26 ( $n=300$ ; 1.7% unknown), with the range from 13 to 63 years old.

From 2014 to 2019, sexual assault cases submitted to HFSC mostly increased per year, but in similar proportion to the total number of requests received. Each year, cases that were presumptive positive for at least one drug other than ethanol remained approximately half (52%–68%). The proportion of confirmed positive cases range from 23% (2016) to 42% (2017). The primary metabolite of cannabis was the most prevalent analyte other than ethanol confirmed in DFSA. It is unknown if the drugs confirmed in the cases were consumed voluntarily or not. The reported demographic data showed the majority of DFSA victims were White females in their mid-20s. The data from this study can aid in raising public awareness about DFSA and in investigations of suspected DFSA in the city of Houston.

### Reference(s):

<sup>1</sup> Texas Department of Public Safety. *Texas Crime Report, 2019*.

### Sexual Assault, Toxicology, DFSA

## K34 Updated Trends and Case Studies in the Lysergic Acid Diethylamide (LSD) Revival

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**Learning Overview:** After attending this presentation, attendees will be familiar with the prevalence and investigative context of LSD in human performance and postmortem casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of ongoing LSD trends and the case details that may indicate hallucinogenic drug usage.

**Introduction:** LSD emerged in the 1960s and lured recreational drug users with its psychedelic and hallucinogenic effects. Its popularity diminished in the following years and remained low for decades. Over the past several years, a revival has taken place and introduced higher potency analogs. Since 2015, the laboratory has witnessed this revival firsthand and reported new case positives for LSD and/or the primary metabolite, 2-oxo-3-hydroxy-LSD, in both blood and urine specimens.

**Methods:** Blood and/or urine specimens for human performance or postmortem casework were screened by an LSD immunoassay kit with a limit of detection of 0.5ng/mL. All positive screens were analyzed on a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) confirmatory method for LSD and its primary metabolite, 2-oxo-3-hydroxy LSD, with linear ranges from 0.05ng/mL to 2.0ng/mL and 0.25ng/mL to 10.0ng/mL, respectively.

**Results:** LSD-positive toxicology cases have been detected in nearly half of the states throughout the country, with the Southeast and Western regions appearing to be the most affected. While most of the cases involve some form of public intoxication, LSD has also been observed as a significant contributor in sexual assault and postmortem cases. Similar to designer benzodiazepines, immunoassays can be a useful tool for detecting emerging LSD analogs. Some analogs such as ALD-52, 1B-LSD, or 1P-LSD yielded presumptive positive results at concentrations as low as 1.0ng/mL whereas AL-LAD, ETH-LAD, and LSZ were observed to have little to no cross-reactivity on the in-house immunoassay, which would lead to possible false-negative casework through this method.

**Conclusions:** While LSD screening may not be warranted in routine panels, case histories can prove especially useful for determining which cases should proceed to screening for LSD or other hallucinogens. When reviewing the history, patient behavior or activities may suggest what type of drug may have been ingested. At a minimum, laboratories should have a validated screening panel for LSD and 2-oxo-3-hydroxy LSD to test casework with suspected hallucinogenic drug use. This can be accomplished using immunoassays, LC/MS/MS, or time-of-flight mass spectrometry. Since not all analogs will cross-react on the immunoassay, it is recommended to utilize a supplemental screen for cases with indicative case histories that confirmed negative for LSD and associated metabolites. As the popularity of LSD and its analogs continue to rise, it is important that labs ensure their testing methods are comprehensive and include LSD and similar compounds. Failure to implement appropriate testing for LSD may result in missed confirmations and reporting cases as falsely negative.

### LSD, NPS, Trends



## K35 Monitoring of Specialized Internet Forums to Ensure Complete Toxicology Testing in Suicide Death Investigations

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**Learning Overview:** After attending this presentation, attendees will have learned about the utility of accessing specialized web-based forums to stay current about real-time drug and substance use trends in the pro-suicide community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how frequent review of member posts on specialized internet forums can be utilized to ensure that suicide death investigations include appropriate toxicology testing and scene investigation.

Suicide is a leading cause of death in the United States. The United States Department of Health and Human Services reported in 2019 that 47,173 people deaths were attributed to intentional self-harm (suicide) and this was the tenth leading cause of death in 2017. Out of these deaths, 6,554 were categorized as Poisoning Suicides.<sup>1</sup> One of the challenges associated with toxicology testing is to ensure that the analysis performed is designed to identify the agents that are commonly used to commit this act. While data monitoring of laboratory findings in this population is one mechanism that can be used for this purpose, inherent risk is associated with this approach. This is because an assessment of this type only provides positive findings and does not include substances that were excluded in the scope of analysis. Another more non-traditional approach is to utilize web-based forums to learn what substances people are planning to use or are inquiring about to commit suicide.

One such forum is called Sanctioned Suicide, which describes itself as “a pro-choice suicide community that discusses mental illness and suicide from the perspective of suicidal people, as well as the moral implications of the act itself.” Forum statistics in October 2020 show 45,838 threads, 853,180 messages, and 16,032 members. Registration is only required to post and send private messages, so forum posts are visible to non-registered users. Sanctioned Suicide has a section dedicated to Methods and another section for Resources; it is these that are frequently evaluated to stay abreast of any new trends or substances that are actively being discussed. At the time of this submission, Sodium Nitrite (SN) is one of the most discussed methods and has its own mega-thread with a detailed “how to” guide. From this example, it is noted that SN is a chemical of interest that would not be identified by routine toxicology testing. Furthermore, members discuss taking an antiemetic such as metoclopramide to prevent vomiting and an antacid to counteract the conversion of SN within the acidic environment of the stomach to a regular salt. This type of surveillance not only provides useful information to toxicology laboratories as they make decisions regarding which tests to develop but is of special relevance to the death investigator. Death investigators will have a greater awareness to look for seemingly innocuous items such as aquarium test strips, which are used to verify the purchased SN contains an appropriate concentration of nitrite.

Suicide is one of the manners of death that can be assigned to a medical examiner or coroner case. It is incumbent upon toxicology laboratories and death investigators to utilize as many avenues as possible, including web-based forums, to ensure a complete and accurate death investigation process.

### Reference(s):

<sup>1</sup>. *FastStats*. August 4, 2020, <https://www.cdc.gov/nchs/fastats/suicide.htm>.

### Suicide, Web-Based Forums, Drug and Substance Use

## K36 Measuring Inhibition of Neurotransmitter Transport *In Vitro* to Predict Effects and Abuse Potential of Novel Cathinone-Type Stimulants

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of *in vitro* assays for stimulants, including what is measured, applicability, and limitations of this type of assay.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with the tools to understand this type of *in vitro* data and correctly implement it in casework.

The pharmacodynamics of stimulant drugs are complex and responsible for the different effect profiles of drugs such as cocaine, amphetamine, and fluoxetine. An important mechanism is the inhibition of Dopamine (DAT), Serotonin (SERT), and Norepinephrine (NET) transporters in the nervous system. The relative preference for inhibiting the different transporters is important for drug effects, and drugs with similar profiles can be expected to have similar effects. Similarly, the relative preference for DAT and SERT has been linked to abuse potential.

A novel assay was developed to measure transport inhibition and characterized seven novel cathinone type stimulants. Stimulants were incubated at 15 different concentrations between 50 $\mu$ M and 3nM for 3h (NET), 6h (SERT) or 10h (DAT) with three different cell lines, each expressing either the DAT, SERT, or NET transporter, and a proprietary dye mix. During incubation, the concentration of fluorescent dye inside the cells increased, while the fluorescence outside the cells was silenced by a quencher unable to enter the cells. The resulting increase in fluorescence was measured and the obtained dose-response curves were used to calculate Inhibitory Concentration 50% (IC<sub>50</sub>) concentrations. The results from eight well-characterized stimulants were compared to literature values. In addition, inhibition profiles of 3F-alpha-PVP, 4Cl-alpha-PVP, alpha-PiHP, MPHP, 4-methylpentedrone, N-ethylnorhexedrone, and N-ethylpentylone (ephylone) were obtained.

All the characterized novel stimulants were most potent at inhibiting DAT and had inhibition profiles similar to that of alpha-PVP. The IC<sub>50</sub> in nM for DAT were 160 (cocaine), 12 (alpha-PVP), 13 (3F-alpha-PVP), 8.0 (4Cl-alpha-PVP), 260 (4-methylpentedrone), 13 (alpha-PiHP), 17 (N-ethylpentylone), 4.5 (MPHP), and 47 (N-ethylnorhexedrone). The IC<sub>50</sub> in nM for SERT were 200 (cocaine), >10,000 (alpha-PVP), >10,000 (3F-alpha-PVP), 1,100 (4Cl-alpha-PVP), 1,300 (4-methylpentedrone), >10,000 (alpha-PiHP), 510 (N-ethylpentylone), 1,900 (MPHP), and 9,000 (N-ethylnorhexedrone). The IC<sub>50</sub> in nM for NET were 560 (cocaine), 66 (alpha-PVP), 33 (3F-alpha-PVP), 70 (4Cl-alpha-PVP), 1,000 (4-methylpentedrone), 150 (alpha-PiHP), 270 (N-ethylpentylone), 86 (MPHP), and 140 (N-ethylnorhexedrone). All stimulants appeared to be full inhibitors except 4-methylpentedrone, which only partially (75%) inhibited SERT transport.

Based on the data from inhibition of DAT, SERT, and NET, it can be expected that the toxicity of most of these novel stimulants is similar to that observed for alpha-PVP. They were most potent in inhibiting DAT, while higher concentrations were needed for NET (2.5–19x higher IC<sub>50</sub> than for DAT) and SERT (30–>830x higher than DAT) inhibition. The exception was 4-methylpentedrone for which a more balanced profile, more similar to that of cocaine, was observed. Also, 4-methylpentedrone was the only partial inhibitor observed in this study. As all stimulants were selective toward inhibiting DAT over SERT, the abuse potential of these novel drugs is expected to be high.

### Potency, NPS, Cathinones

## K37 Brain Concentrations of 3,4-Methylenedioxypropylamphetamine (MDPV) and Its Metabolites in Male Rats: The Relationship to Pharmacodynamic Effects

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge in how the synthetic cathinone MDPV and its metabolites distribute in the brain and the pharmacological effect of these substances on the rat locomotor behavior, temperature, and postmortem neurochemistry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the competence and performance of attendees in toxicological tools for the analysis of synthetic cathinones and metabolites in plasma and brain samples and the pharmacological interpretation of the analytical results.

**Background:** MDPV is a novel stimulant belonging to the synthetic cathinone class, which has caused severe intoxications and deaths. MDPV mimics the effects of other stimulants such as cocaine and methamphetamine, and possesses a high potential for abuse. Acquisition of preclinical data about MDPV pharmacokinetics is lacking, especially with regard to brain concentration of the drug and its metabolites.

**Objectives:** The goal of the present study was two-fold: (1) to determine the brain concentrations of MDPV and its two major metabolites, 3,4-hydroxypropylamphetamine (3,4-catechol-PV) and 4-hydroxy-3-methoxy-propylamphetamine (4-OH-3-MeO-PV), after systemic administration of MDPV to rats; and (2) to relate brain kinetic parameters to MDPV-induced locomotor activation, body temperature, and postmortem neurochemistry.

**Methods:** Male Sprague-Dawley rats (300–400g) received subcutaneous (s.c.) injections of MDPV (1, 2, or 4mg/kg) or its saline vehicle ( $n=6$ /dose group). Groups of rats were decapitated at 40min and 240min post-injection, and trunk blood and brains were collected. Blood was centrifuged to obtain plasma, whereas brains were dissected to obtain prefrontal cortex, frontal cortex, and dorsal striatum. Plasma and brain tissue were stored at  $-80^{\circ}\text{C}$  until analysis. Just prior to decapitation, rats were observed and rated for locomotor behavior using a numerical score, and core temperature was taken using a rectal probe. Plasma and prefrontal cortex were analyzed by liquid chromatography/mass spectrometry to determine concentrations of parent drug and its metabolites. Frontal cortex and striatum were analyzed by high-pressure liquid chromatography/electrochemical detection to determine concentrations of Norepinephrine (NE), Dopamine (DA), and Serotonin (5-HT). All statistical analyses were performed with GraphPad Prism.

**Results:** Brain and plasma concentrations of MDPV increased with increasing the dose administered. This rise was dose-proportional up to 2mg/kg dose but showed non-linear accumulation at 4mg/kg. The ratio of brain-to-plasma analyte concentration was determined at both time points and at the three different doses. In all cases, MDPV showed brain-to-plasma ratios much greater than 1 (8.8–12.1), whereas 3,4-catechol-PV and 4-OH-3-MeO-PV showed brain-to-plasma ratios much less than  $<1$  (0–0.3). MDPV induced dose-related increases in locomotor activation at 40 and 240min, and a delayed increase in body temperature. MDPV produced a dose-related increase in cortical NE, but only at the 40min time point ( $p<0.05$ ). Brain MDPV concentrations were significantly correlated with locomotor activity but not changes in body temperature or postmortem neurochemical measures.

**Conclusion/Discussion:** MDPV displays linear pharmacokinetics in plasma and brain at 1 and 2mg/kg doses, but non-linear accumulation at 4mg/kg. MDPV freely crosses the blood-brain barrier, but its hydroxylated metabolites do not. MDPV metabolites are more polar and conjugated, which may impede brain penetration. Targeting the glucuronide metabolites in plasma could be more useful than targeting MDPV to extend the window of detection in drug testing. Despite its well-known inhibition of DA uptake, MDPV had no effect on brain tissue DA. MDPV caused a small increase in cortical NE, but this effect was transient. In summary, these findings show that MDPV-induced behavioral effects are related to brain concentrations of the parent compound and not its metabolites. However, the lack of effect of MDPV on monoamine systems suggests other mechanisms may contribute to effects of the drug *in vivo*.

### MDPV, Pharmacokinetics, Pharmacodynamics

## K38 A Metabolic Profile Determination of 2F-Viminol, A Novel Synthetic Opioid (NSO) Identified in Forensic Investigations

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**Learning Overview:** After attending this presentation, attendees will know about the proposed metabolites of 2F-viminol through the visualization of elucidated metabolite structures and metabolic pathways that were determined from Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) data obtained through analysis of human liver microsome incubations of the drug.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about an NSO with limited pharmacological characterization. The findings will also provide laboratories with biomarker information for the identification of this drug in toxicological samples, such as blood or urine.

Although minor progress has been made in the opioid epidemic, opioid-related deaths in many parts of the United States continue to rise or remain high as synthetic opioids become more prevalent. NSOs, including various fentanyl analogs as well as emerging non-fentanyl-derived compounds, have been identified in products for stand-alone use, as constituents of counterfeit preparations, and as adulterants in other common opioid products such as heroin. A particular new NSO, 2F-viminol, has been identified in casework at the Center for Forensic Science Research and Education through their Novel Psychoactive Substances (NPS) Discovery Program. Thus far, little information and published literature are available for this new opioid. Viminol, a previously developed opioid, is structurally similar and is chlorinated at the 2-position. Despite how structurally different viminol is from other subclasses of opioids, studies have shown it to display significant analgesic and pharmacological properties, comparable to those of morphine. The replacement of chlorine with fluorine on the molecule could make 2F-viminol a more potent drug than viminol, with considerable toxicity due to the prolonged half-lives and increased lipophilicity of fluorinated drugs; however, studies will need to be conducted to confirm this information.

Studying the metabolism of NSOs is crucial to identifying the products of biotransformation that a drug undergoes in the body after ingestion. Currently, there is no literature available with information regarding the metabolism of 2F-viminol. To address this, Human Liver Microsomes (HLM) were used to perform *in vitro* metabolism studies using a drug standard. The goal was to predict and confirm its *in vivo* metabolism. Experimental samples prepared using HLMs and 2F-viminol were analyzed via a SCIEX™ TripleTOF® 5600+ LC/qTOF/MS. The generated metabolic structures were elucidated using SCIEX™ MetabolitePilot™ software (version 2.0). Data features evaluated included formula, accurate mass and mass error, retention time, fragment data, and a proposed structure. After final data review, the primary metabolite(s) of 2F-viminol can then be confirmed in authentic samples and added to screening protocols, helping to extend the window of detection for the parent drug in toxicological samples. Overall, at least three metabolites of 2F-viminol were discovered, including N-dealkylated species.

### Novel Synthetic Opioid, Metabolism, Toxicology

## K39 Long-Term Stability Assessment of Fentanyl Analogs in Blood Using 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 stability of fentanyl analogs in blood specimens over a nine-month time frame.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a novel fentanyl analog quantification method and knowledge of fentanyl analog stability.

Fentanyl analogs played a major role in proliferating the opioid epidemic in the United States. While fentanyl analog use has recently decreased due to regulation, overdose deaths caused by these drugs are not uncommon. With high rates of overdose deaths, forensic toxicology laboratories often experience backlogs, resulting in extended sample storage time. To address this issue, a quantitative method was developed and validated for fentanyl analogs in blood using LC/qTOF/MS. The method was then applied to a long-term stability study (nine months) to assess fentanyl analogs in blood at various temperatures.

Analytes of interest were methoxyacetylfentanyl, acetylfentanyl, acrylfentanyl, 4-ANPP, fentanyl, furanylfentanyl, p-fluorofentanyl, cyclopropylfentanyl, 3-methylfentanyl, carfentanil, butyrylfentanyl, 4-fluoro-isobutyrylfentanyl, and valerylfentanyl. Blood samples (250µL) were subjected to a previously published solid-phase extraction procedure. Quantification was performed using an Agilent® Technologies 1290 Infinity liquid chromatograph coupled to an Agilent® Technologies 6530 Accurate Mass TOF/MS operated in targeted acquisition mode.

For long-term stability, potassium oxalate and sodium fluoride-preserved blood (100mL) was fortified at a low (10ng/mL) and a high (80ng/mL) concentration, and aliquots were subjected to the following conditions: frozen (-20°C), refrigerated (4°C), room temperature (~25°C), and elevated temperature (35°C). All samples were stored in glass vacutainer tubes, except frozen, which were stored in plastic falcon tubes. Samples were analyzed fresh ( $t_0$ ) and after 24h, 48h, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and then monthly (for 9 months). Stability samples were extracted and analyzed using the present validated method. Analyte concentrations were compared to  $t_0$ , and analytes were considered stable if compounds quantified within  $\pm 20\%$ .

The method was validated according to American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036. Calibration ranges were 1–100ng/mL for all analytes. Limits Of Detection (LOD) were 0.5ng/mL for all analytes. Precision and bias values were determined to be acceptable for all analytes (within  $\pm 7.2\%CV$  and  $\pm 15.2\%$ , respectively). Matrix effects exhibited minor ion enhancement for all analytes, except carfentanil and 4-ANPP in the low quality control (ion enhancement  $>25\%$ ). No carryover, endogenous, or exogenous interferences were encountered. Short-term stability studies were performed to establish time points for the long-term portion of the study. All analytes remained stable under the following conditions: 48h in the autosampler, 24h room temperature, and 72h refrigerated. Acrylfentanyl was unstable at room temperature ( $>\pm 20\%$  bias).

Over the first three months, the fentanyl analogs of interest (except acrylfentanyl) remained stable under room temperature and refrigerated conditions at both concentrations (84.0%–112.5%). While most fentanyl analogs remained stable under frozen conditions, downward trends were observed after two weeks (4 freeze/thaw cycles). At elevated temperatures, most analytes were stable for one week at both concentrations (74.2%–112.6%). Acrylfentanyl was determined to be unstable after 24h under elevated ( $<30\%$ ) and room temperature ( $<50\%$ ) conditions, after 48–72h under refrigerated (59.5%–82.0%) conditions, and four weeks under frozen (73.0%–78.0%) conditions.

This research presents a validated method for the quantification of fentanyl analogs in blood using LC/qTOF/MS and has determined analyte stability in blood at four temperatures over the first three months of an in-progress nine-month study. While fentanyl analogs are observed stable for months under various temperature conditions, optimal storage is recommended under refrigeration. In the event of improper storage or handling, subjecting samples to elevated temperatures may remain stable for about one week. However, acrylfentanyl was determined to be extremely unstable and immediate analysis would be required for a suspected case.

### Fentanyl Analogs, Postmortem Blood, Stability



## K40 Quantitative Forensic Toxicology by Standard Addition: Consideration, Experimentation, and Implementation

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**Learning Overview:** After attending this presentation, attendees will be able to assess the value of standard addition in comparison to traditional external calibration approaches and be able to implement a standard addition protocol in their laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding knowledge of types of acceptable quantitative analyses and by providing an avenue that is effective and efficient with the possibility of changing forensic quantitative approaches in the future.

Quantitative determination of drug concentrations in forensic toxicology samples, especially blood, can assist a forensic toxicologist with interpretation of analytical findings in the context of an investigation. Knowing a drug concentration allows a forensic toxicologist to compare results from a specific sample or case to previously published literature or *per se* limits (e.g., blood ethanol concentrations). Most commonly, quantitative forensic toxicological analyses are performed using external calibration curves and control samples to establish a concentration gradient and to test the accuracy and precision of the analytical process, respectively. The Academy Standards Board (ASB) has adopted a standard practices document for evaluation of this type of calibration model through method validation. This process generally takes at least five days to complete a long series of experiments to prove a developed method is acceptable for quantification of a drug in a specific matrix and within a certain concentration range. However, the use of an external calibration model and validation of that process may not be the most effective, efficient, or cost-conscious approach depending on the frequency with which a drug is detected, its prevalence among given populations, and/or its life cycle.

Standard addition is an alternative quantitative approach that has been used for many years among various analytical fields for determination of concentration for a species in a matrix. Standard addition has been previously used in the field of forensic toxicology; however, its current implementation is scarce, and many scientists are not familiar with the approach. The method of standard addition uses an internal calibration model where drug standard is fortified to case samples aliquoted in replicate. Comparison of spiked concentration and Peak Area Ratios (PAR) between analyte and internal standard allow for the analyst to calculate the concentration of drug in the unfortified sample. There are several pros and cons to using standard addition over traditional calibration curves, which will be discussed during this presentation.

The method of standard addition can scientifically be applied to various drug classes and matrix types but lends itself especially useful for quantification of emerging Novel Psychoactive Substances (NPS), since analytical methods typically do not exist for these drugs and their lifespan can be quite short. The laboratory has employed standard addition for the quantification of several different NPS over the past two years. In each scenario, an analytical method was developed using a Waters® Xevo-TQS® micro Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS). Drugs were extracted from matrix by Liquid-Liquid Extraction (LLE) using borax buffer (0.1M, pH 10.4) and N-butyl chloride and ethyl acetate (70:30, v:v). Prior to testing of authentic case samples, the analytical method and standard addition approach were verified using a series of experiments adopted from the ASB standard. Experiments assessed linearity over the target range, limit of detection, recovery, and interference from matrix, analyte, internal standard, and commonly encountered drugs, as well as mock standard addition on fortified control samples.

For quantification, four replicate samples were aliquoted and prepared by fortification with drug standard: one sample remained “blank” with no drug standard added and three samples were “up-spiked” at appropriate concentration for the specific drug. After LC/MS/MS analysis, resulting analyte-internal standard PAR were plotted against the up-spike concentration. A linear trendline between all data points was implemented. Correlation ( $R^2$ ) between the data points was required to be greater than 0.98. The concentration of drug in the sample was determined by calculating the x-intercept of the plotted line.

This approach has been successfully implemented for the quantitative determination of NPS in forensic casework, including isotonitazene (opioid), bromphine (opioid), eutylone (stimulant), 2F-deschloroketamine (hallucinogen), hydroxy-PCP (hallucinogen), and flualprazolam (benzodiazepine). Specific drug and case results will be discussed further during this presentation. The laboratory has had overwhelming success with standard addition. It is recommended that forensic toxicologists consider standard addition as an acceptable alternative quantitative approach for certain drugs and sample types, when appropriate.

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### Forensic, Toxicology, Quantitation

## K41 The Quantification of Oxycodone and Its Phase I and II Metabolites in Urine

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the significance of incorporating phase II metabolites into methods to investigate oxycodone use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by filling a knowledge gap regarding the excretion of oxymorphone-3 $\beta$ -D-glucuronide and noroxymorphone-3 $\beta$ -D-glucuronide after controlled dosing and their presence in case samples.

The purpose of this research was to develop and validate a comprehensive analytical method for the detection and quantification of noroxymorphone-3 $\beta$ -D-glucuronide, oxymorphone-3 $\beta$ -D-glucuronide, noroxymorphone, oxymorphone, 6 $\alpha$ -oxycodol, 6 $\beta$ -oxycodol, noroxycodone, and oxycodone in urine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) primarily to be used in a human study.

An ACQUITY® UPLC® I-class coupled to a Waters® Xevo-TQD® was used for analysis. Separation was achieved using an ACQUITY® HSS T3 column (1.7 $\mu$ m, 2.1 x 100mm) at 30°C at 0.5mL/min. Mobile phase A was 0.001% formic acid in 10mM ammonium formate (pH 5.2) and mobile phase B was 0.001% formic acid in acetonitrile. The gradient started with 2% mobile phase B for 1.5min, then increased to 25% for the next 4.7min, and ended at 7.1min after high organic wash and re-equilibrium. Two transitions were monitored for each analyte and one for the deuterated internal standards. The method was validated according to the Academy Standards Board (ASB) Standard Practices for Method Development in Forensic Toxicology. The method was then applied to a single-dose pilot study of a subject. Urine samples were collected from the subject after ingesting 10mg oxycodone as an immediate release tablet. The time of collections were 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 24, 48, 72, and 96 hours. Additionally, urine specimens ( $n=15$ ) from petty drug offences that had previously been confirmed positive for oxycodone were analyzed using the newly validated method.

The calibration range for noroxymorphone-3 $\beta$ -D-glucuronide and oxymorphone-3 $\beta$ -D-glucuronide was 0.05–10 $\mu$ g/mL, while the calibration range for all other analytes was 0.015–10 $\mu$ g/mL. The standard curves for all analytes were fitted using a linear regression with a 1/x weighting within the calibration range, except for noroxymorphone and 6 $\alpha$ / $\beta$ -oxycodol, which were determined using a quadratic regression with a 1/x weighting. The limits of quantification were determined to be 0.015 $\mu$ g/mL for oxycodone, noroxycodone, 6 $\alpha$ / $\beta$ -oxycodol, oxymorphone, and noroxymorphone and 0.050 $\mu$ g/mL for noroxymorphone-3 $\beta$ -D-glucuronide and oxymorphone-3 $\beta$ -D-glucuronide. Imprecision ranged between 1.8 and 13%CV. Bias was determined to be between -12.2% and -0.4%. Process efficiency was within  $\pm 25\%$  for all analytes except for noroxymorphone-3 $\beta$ -D-glucuronide (71%). No carryover was observed after the injection of the highest calibrator (10 $\mu$ g/mL). Dilution integrity was acceptable for a ten-fold dilution after analytes quantified within an acceptable limit (98%–111%) of the target concentration.

After the method was validated, urine samples from the pilot study ( $n=1$ ) were analyzed. Urine concentrations were corrected for creatinine concentration. It was observed that oxycodone, noroxycodone, and oxymorphone-3 $\beta$ -D-glucuronide contained the highest concentrations and were present in either the 0.5h or 1h void. Noroxycodone and oxymorphone-3 $\beta$ -D-glucuronide were detected until the 48h sample, while oxycodone was only detected up to the 24h sample.  $T_{max}$  in urine was achieved within 1.5h for oxycodone and with 3h for noroxycodone and oxymorphone-3 $\beta$ -D-glucuronide.  $C_{max}$  in urine for oxycodone, noroxycodone, and oxymorphone-3 $\beta$ -D-glucuronide was 3.15, 2.0, and 1.56 $\mu$ g/mg, respectively. From the authentic urine specimens, oxycodone concentrations ranged from 0.015–12 $\mu$ g/mL. Ranges for noroxymorphone-3 $\beta$ -D-glucuronide and oxymorphone-3 $\beta$ -D-glucuronide were 0.054–9.7 $\mu$ g/mL and 0.14–67 $\mu$ g/mL, respectively. It was observed that larger concentrations of oxycodone did not always result in larger concentrations of the phase II metabolites. In the future, the excretion of phase I and II metabolites in a controlled dosing study involving both immediate and extended release formulations of oxycodone will be investigated.

A comprehensive method for the quantification of noroxymorphone-3 $\beta$ -D-glucuronide, oxymorphone-3 $\beta$ -D-glucuronide, noroxymorphone, oxymorphone, 6 $\alpha$ -oxycodol, 6 $\beta$ -oxycodol, noroxycodone, and oxycodone in urine was optimized and met validation criteria. Including noroxycodone and phase II metabolites of oxycodone benefited analyses by extending the window of detection. The concentrations of noroxymorphone-3 $\beta$ -D-glucuronide and especially oxymorphone-3 $\beta$ -D-glucuronide presented in this study provide details needed in the forensic community to better comprehend oxycodone pharmacokinetics.

### Oxycodone, Pharmacokinetics, Oxymorphone Glucuronide

## K42 Chiral Separation and Analysis of Methylphenidate, Ethylphenidate, and Ritalinic Acid in Blood by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** After attending this presentation, attendees will understand chiral separation and method validation of cognitive stimulants and how to effectively analyze them on an LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with a unique approach to the extraction of cognitive stimulants from blood. With this, a novel approach to chiral separation and analysis 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 isomers, threo and erythro, though the erythro isomer is the only one proven to give pharmacological effects. The erythro isomer is present as Dextro (D) or Levo (L) configuration, with the D configuration being more potent. However, many medications are sold as a racemic mixture. This makes analytic separation of the isomers essential. MPH metabolizes into Ritalinic Acid (RA) as well as Ethylphenidate (EPH) in the presence of ethanol. Chiral analysis poses challenges to researchers. Currently, there are no methods available to detect these chiral stimulants in a single analysis. Due to limited assays, this project aimed to develop a method that separates and quantifies the enantiomers of MPH and EPH as well as RA following isolation and extraction from blood samples. Methods such as this are critical to understanding the pharmacokinetics of such cognitive stimulants. The goal of this study was to fully develop and validate a method to separate the threo-enantiomers of d,l-MPH, d,l-EPH, and RA and quantify them in blood utilizing LC/MS/MS.

MPH, EPH, and RA were extracted from blood (250 $\mu$ L) using Solid Phase Extraction (SPE). Blood was fortified with calibrator or control solution (25 $\mu$ L) and internal standard (25 $\mu$ L), mixed with phosphate buffer (1mL, pH 6, 100mM), then centrifuged. The supernatant was loaded onto an UCT Clean Screen<sup>®</sup> DAU column (130mg/3mL) on an SPE cartridge previously conditioned with methanol and phosphate buffer. Following washes with 0.1M acetic acid and methanol, compounds were eluted with 2% ammonium hydroxide in methanol. Analysis was performed on an AGILENT<sup>®</sup> 1290 Infinity Liquid Chromatograph coupled to an AGILENT<sup>®</sup> 6470 Triple Quadrupole MS. Separation was achieved using an AGILENT<sup>®</sup> Chiral-V column (2.7 $\mu$ m, 2.1x100mm) with an isocratic elution of 2:98 mobile phase A:B at 0.6mL/min. Mobile phase A was deionized water and mobile phase B was 0.025% ammonium acetate and 0.0125% trifluoroacetic acid in methanol. A Multiple Reaction Monitoring (MRM) method was used to detect the analytes with one transition for quantification and one for qualification. The method was validated according to Academy Standards Board (ASB) guidelines, including: precision and bias, linearity, carryover, interferences, matrix effects, Limit Of Detection (LOD), Limit Of Quantification (LOQ), dilution integrity, and stability.

The linear range for MPH and EPH was 0.5–200ng/mL and 0.5–500ng/mL for RA ( $R^2 > 0.99$  for five days). LOD was determined to be 0.25ng/mL for all analytes and LOQ was 0.5ng/mL. Extraction recovery was >79%. Matrix effects were determined via post-extraction addition and displayed enhancement for MPH and EPH (17%–58%) and suppression for RA (51%–58%). Bias ranged from -12.7% to -4.8% and maximum within run precision was 12.5% for all analytes. Stability was evaluated as a processed sample stability in the autosampler (48h, 4°C), refrigerated (48h, 4°C), and at room temperature (24h, 24°C), and all analytes were considered stable and quantified within 16%. No carryover was observed. Endogenous and exogenous interferences were evaluated and had no significant impact.

This method was developed and fully validated for the quantification of d,l-MPH, d,l-EPH, and RA in blood. This is the first method, per research, that successfully separates the enantiomers and quantifies all analytes within a single analysis without the use of chiral derivatization. Due to differing effects of these enantiomers as well as different rates of metabolism, it is essential to separate these compounds. This method can be utilized to help better understand the pharmacokinetics of these chiral stimulants.

### Methylphenidate, Chiral Analysis, LC/MS/MS

## K43 Cannabis and Driving Throughout the Legalization Process: A Portrait of the Province of Québec, Canada

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**Learning Overview:** The goal of this study is to provide an overview of Tetrahydrocannabinol (THC) in Driving Under the Influence of Drugs (DUID) cases in the context of recreational cannabis legalization in Canada. Data on prevalence, blood concentrations, and concomitant use of other drugs will be reviewed.

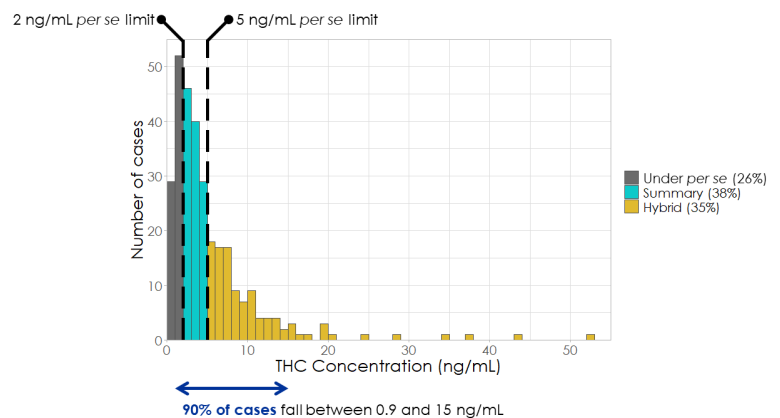
**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data on the changes that can occur in DUID casework upon legalization of cannabis and modifications to the drugs and driving legislation. Furthermore, THC blood concentrations of drug-impaired drivers will be provided, contributing to the discussion about legal thresholds for this substance.

**Introduction:** On October 18, 2018, Canada legalized recreational consumption of cannabis. Prior to this legalization, on June 26, 2018, new legislation modified the modalities surrounding driving under the influence offences. Notably, legal thresholds were introduced for ten drugs; for THC, these *per se* limits are established at 2ng/mL (summary offense, less serious nature) and 5 ng/mL (hybrid offense). Furthermore, legal requirements for blood collection were softened, now allowing police officers to require a blood sample by demand based on reasonable grounds instead of requiring a warrant to do so. This study reviews findings in drugged drivers where analysis of the blood sample submitted revealed the presence of THC.

**Method:** Blood samples were systematically analyzed for cannabinoids and more than 180 drugs and metabolites (including, for example, benzodiazepines, amphetamines, and opioids) by Liquid Chromatography coupled to Tandem Mass Spectrometry (LC/MS/MS). This targeted method, relying on a protein precipitation extraction, acted both as a screening and confirmation method. Concentration reporting limits used were 0.5ng/mL for THC and 5ng/mL for its inactive metabolite, 11-nor-9-Carboxy- $\Delta$ -9-Tetrahydrocannabinol (THC-COOH). Ethanol analysis was performed only if elements in the police investigation warranted it. For all driving under the influence cases tested by the laboratory, data related to drivers (age, gender), the arrest (date, time, location, investigative tools), the sample(s) (collection date and time, type of biological matrix), and findings (detected analytes and concentrations) were compiled using a Microsoft® Excel® database. Summary statistics and data visualization were generated using Excel®, R, and RStudio®.

**Results:** Between January 1, 2018, and August 1, 2020, 896 whole blood DUID samples were analyzed. THC was detected in 329 of these (37%). THC prevalence in blood casework (normalized to take into account the unequal time frames) significantly increased after legalization, going from 27% to 39% (two-proportion z-test,  $p = 0.0098$ ). The distribution of THC concentrations is shown in the figure below (median 3.6 ng/mL) and did not change significantly over the studied period. In 76% of cases, other psychoactive drugs were detected, mainly methamphetamine (40%), cocaine (12%), Gamma-Hydroxybutyrate (GHB, 11%), and synthetic benzodiazepines (10%). Ethanol analysis was performed in 47% of cases and was found positive in 24% of these cases (median 86mg/100 mL, range 10–258).

**Conclusion:** Following cannabis legalization, a significant increase of THC prevalence in whole blood DUID was observed, which could be due to a variety of factors concurrent with legalization and legislation. Hypothetical causes would include, for example, an increase in the proportion of drivers under the influence of cannabis, better and more systematic use of standardized field sobriety tests and greater effectiveness in detecting cannabis users among drugged drivers. However, these conclusions cannot be directly extrapolated to cannabis and driving in general, since DUID cases with blood collection remains a small fraction of overall DUIDs in the province of Quebec, with urine following a Drug Recognition Expert (DRE) evaluation remaining the main DUID investigation tool to this day.



### DUID, THC, Legalization

## K44 Updated Techniques for Characterizing Cannabis Use

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**Learning Overview:** The goals of this presentation are to raise awareness on the emergence of several phytocannabinoids observed in current forensic casework and describe techniques to identify and confirm these compounds in human specimens using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that traditional analytical methods may require adjustments to achieve adequate resolution between cannabinoid isomers. It is important to monitor changes to cannabis legislation and adapt analytical methods accordingly.

**Background/Introduction:** Cannabis use continues to be a growing problem for forensic investigations. Although marijuana is still illegal federally, decriminalization, especially at the state-level, has permitted the widespread accessibility of cannabis. Cannabis is now available in several forms such as conventional marijuana, extracts, and edible products containing Cannabidiol (CBD) from hemp. In the military, a zero-tolerance stance remains for using cannabis products, including CBD, primarily because CBD products are unregulated. Dronabinol is the Food and Drug Administration (FDA) - approved form of pure  **$\Delta$ -9-Tetrahydrocannabinol ( $\Delta$ -9-THC)**, which is allowed in military medical care, but also may be used to mask surreptitious use of cannabis products. Therefore, distinguishing dronabinol from cannabis use is required in some cases. Further, new cannabis strains and processing methods have led to the emergence of products containing increased  **$\Delta$ -8-Tetrahydrocannabinol ( $\Delta$ -8-THC)** content. These issues have motivated renovations in approaches to enhanced screening and confirmation methods regarding cannabis toxicology analyses.

**Objectives:** This presentation raises awareness on the emergence of several phytocannabinoids observed in current forensic casework and describes techniques to identify and confirm these compounds in human specimens using LC/MS/MS.

**Methods:** Blood and/or urine specimens were submitted to the laboratory as investigative cases. Routine screening encompassed a drugs of abuse immunoassay for nine drug classes, an alcohol screen by headspace gas chromatography, and a basic drug screen. If case history indicated potential CBD use, a phytocannabinoid LC/MS/MS screen was utilized to monitor for CBD, its metabolite 7-carboxy cannabidiol, cannabigerol, cannabinol,  $\Delta$ -9-Tetrahydrocannabinol (THCV), and 11-nor-9-carboxy-THCV. Presumptive positive screens were confirmed by LC/MS/MS analysis with a limit of detection at 1.0ng/mL for non-carboxylated analytes and 5.0ng/mL for carboxylated analytes. If an analyst detected possible  $\Delta$ -8-THC or 11-nor-9-Carboxy- $\Delta$ -8-Tetrahydrocannabinol ( $\Delta$ -8-THC-COOH) presence during confirmative LC/MS/MS analysis for  $\Delta$ 9-THC and its metabolites, an additional extraction was performed to confirm the presence of  $\Delta$ -8-THC and  $\Delta$ -8-THC-COOH by LC/MS/MS.

**Results:** An enzyme hydrolysis step yielded ten-fold higher signal for CBD in authentic human samples as compared to conventional alkaline hydrolysis. This improvement in CBD-glucuronide analysis has previously been attributed to enhanced cleavage of the ether glucuronide. When analyzing case specimens, the  $\Delta$ -9-THC and metabolites method displayed chromatographic shouldering on the  $\Delta$ -9-THC and  $\Delta$ -9-THC-COOH peaks. These interfering peaks were identified with standard reference materials as  $\Delta$ -8-THC and  $\Delta$ -8-THC-COOH, respectively. This analytical method was adjusted to achieve adequate separation between these isomers. Among several of the cases analyzed during this time, one of them was below the limit of detection for both  $\Delta$ -9-THC and  $\Delta$ -9-THC-COOH, yet above the limit of detection for  $\Delta$ -8-THC and  $\Delta$ -8-THC-COOH. Without these improvements, this case would have been reported as negative if the additional  $\Delta$ -8-isomer testing had not been pursued.

**Conclusion/Discussion:** Because of allowances under the 2018 Farm Bill, products containing CBD and other cannabinoids have been readily available for consumption. Standard screening procedures are unable to detect many of these phytocannabinoids; therefore, more targeted screening methods must be implemented in order to properly identify these compounds in human specimens. Traditional analytical methods may require adjustments to achieve adequate resolution between cannabinoid isomers. It is important to monitor changes to cannabis legislation and adapt analytical methods accordingly.

### Cannabis, LC/MS/MS, Screening

## K45 A Determination of $\Delta$ -9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in Edibles Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Overview:** The goal of this presentation is accurately determining the amount of CBD and THC from edibles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community for accurately analyzing the abuse drug by quantitatively determining the amount of CBD and THC in edibles from local stores. This presentation will also help to establish a reliable method to determine CBD and THC to promote the research and development of industrial hemp.

Cannabis products are by far the most abused drugs on the illicit drug market. The most popular used cannabis products are  $\Delta$ -9-THC and CBD. Tetrahydrocannabinol Acid (THCA-A) is a non-intoxication cannabinoid and as the plant dries, this gets transformed into THC by decarboxylation as heat expedites. Cannabinol (CBN) is a lesser-known cannabinoid that is less than 1% in most strains, but it could be generated when THC is oxidized.

Several states had legalized the use of medical cannabinoids as well as the recreational use. The most common way of administering cannabinoids is the oral route, in the form of edibles, such as baked goods, candies, gummies, chocolates, and beverages. Because it takes longer for the initial psychoactive effect of edibles to be felt, the edible could be easily overconsumed. Overdosing on cannabinoids might cause severe health and mental problems. On the other hand, there is a possibility that the labeled amounts of CBD and THC do not reflect their real values, and the residues of THCA-A and CBN in the edibles could be another concern. However, determination of cannabinoids in edibles is a problematic task due to the complexity of the involved matrices. Therefore, a reliable and accurate method to determine CBD, THC, THCA-A, and CBN is needed. In this study, a sensitive LC/MS/MS was developed to determine active cannabis compounds from edibles.

Edible samples were purchased from two CBD shops in Emporia, KS, including gummy bears, chocolate sandwich cookies, chocolate bars, honey sticks, and CBD water. For sample process, gummy bears, cookies, and chocolate bars were cut into small pieces, frozen overnight, and ground to powder. One gram of sample was soaked with 10mL water for 30 minutes, 10ml of acetonitrile was added, extracted with Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) salt, purified by Clean Screen<sup>®</sup> THC extraction Solid-Phase Extraction (SPE) column, then analyzed with LC/MS/MS. For the honey stick and water sample, 1mL sample was diluted with CAN and directly analyzed with LC/MS/MS.

The recovery study was conducted with similar blank samples (gummy bears, Oreo<sup>®</sup> sandwich cookies purchased from a local Walmart<sup>®</sup> store). THC, THCAA, CBN, and CBD standards were spiked into the sample, then extracted and analyzed with LC/MS/MS. THC, THCA-A, CBN, and CBD were separated on a Agilent<sup>®</sup> Poroshell 120 EC-C<sub>18</sub> column, and detected by triple-stage quadrupole mass spectrometer with linear ion trap capability (SCIEX<sup>™</sup> 3200 Qtrap<sup>®</sup>). THC-d<sub>3</sub> and CBD-d<sub>3</sub> were used as internal standards. THC, CBN, and CBN were analyzed in positive mode and THCA-A was analyzed in negative mode.

Linearity, sensitivity, matrix effects, and recovery were studied to validate the method. The results show the method is satisfied for quantitatively analyzing THC, THCCA, CBN, and CBD from edibles. The amount of those four cannabinoids in the edible will be determined by the method and provide the accurate information of its content.

### THC, CBD, Edibles

## K46 The Prevalence of Cannabidiol (CBD) and Tetrahydrocannabinol (THC) in Federally Regulated Workplace Drug Testing Urine Specimens

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**Learning Overview:** The goals of this presentation are for attendees to understand the prevalence of THC, CBD, and their metabolites in federally regulated workplace specimens and to relate the prevalence of these analytes in workplace drug testing specimens to the consequence of passage of the 2018 Federal Farm bill.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the impact of federal legalization of hemp containing <0.3% THC on federally regulated workplace urine drug testing results.

**Background/Introduction:** The non-psychoactive cannabinoid, CBD, is marketed as a remedy for a wide array of medical conditions. Consequently, there is growing use of CBD-containing products by the general public that may have unintended consequences to the consumer. Of concern is that CBD supplements are not subject to active regulatory oversight so their composition may deviate widely from the products' labelling and may contain  $\Delta$ -9-THC, a psychoactive component of the cannabis plant. This puts the consumer in a buyer beware situation and presents a dilemma to the laboratory or medical review officer if the donor asserts legal CBD use as the reason for a positive drug test. To better understand the prevalence of THC, CBD, and their metabolites in federally regulated workplace specimens, a pulse testing study of 2,000 urine specimens was undertaken.

**Objective:** Determine the positivity rate of 11 cannabinoids and cannabinoid metabolites in regulated workplace drug testing specimens by screening and confirmation methods.

**Methods:** In the normal course of workplace testing, specimens are aliquoted into test tubes and loaded onto automated instruments for screening by Immunoassay (IA); after review, these aliquots are discarded. This study utilized 2,000 de-identified urine aliquots scheduled for discard to screen for 7-Hydroxy Cannabidiol (7-OH-CBD) and 11-nor-9-Carboxy- $\Delta$ -9-Tetrahydrocannabinol ( $\Delta$ -9-THC-COOH) using a fast Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technique with a 2ng/mL cutoff. Further, specimens positive using the fast chromatographic screen were analyzed by IA for THC-COOH at 20, 50, and 100ng/mL cutoffs. Positive specimens were then confirmed for THC, CBD, and nine other cannabinoids and/or metabolites by LC/MS/MS following sequential enzymatic and basic hydrolysis.

**Results:** Of the 2,000 urine specimens initially screened by fast LC/MS/MS (Limit of Detection [LOD]=2ng/mL) for 7-OHCB and  $\Delta$ 9-THCCOOH, 186 (9.3%) specimens were identified and further analyzed by LC/MS/MS. The percent samples (based on  $n=2,000$ ) that screened positive by IA were as follows: 20ng/mL cutoff, 1.45%; 5 ng/mL cutoff, 0.9%; and 100ng/mL cutoff, 0.55%. The percent prevalence of the 11 analytes (LOD=1ng/mL) in order of highest abundance was as follows:  $\Delta$ -9-THC-COOH, 4.9%; 11-OHTHC, 2.85%; 7-OHCB, 2.8%; CBDA, 1.7%; CBD, 1.55%; THCV, 0.9%;  $\Delta$ -9-THC, 0.45%;  $\Delta$ -8-THC-COOH, 0.4%; CBN, 0.15%; and THCV, 0.05%. Five specimens screened positive by IA (20ng/mL cutoff) but tested negative (<15 ng/mL) for  $\Delta$ -9-THC-COOH. Of these, one specimen contained 41.0ng/mL of  $\Delta$ -8-THC-COOH and 1.9ng/mL of  $\Delta$ -9-THC-COOH.

Specimens confirming positive for one or more studied analytes ( $n=137$ ) are ranked by CBD and combinations of CBD analytes as follows: all negative, 56.2%; positive CBDA/7-OHCB/CBD, 17.5%; positive 7-OHCB, 15.3%; positive CBDA/7-OHCB, 5.1%; positive 7-OHCB/CBD, 3.6%; positive CBDA/CBD, 1.5%; and positive CBDA, 0.7%.

**Conclusions:** A total of 98 (4.9%) specimens had  $\Delta$ -9-THC-COOH concentrations  $\geq 1$ ng/mL and 60 (3.0%) specimens contained CBD and/or CBD metabolites. It is unsurprising that THC analyte-positive specimens were also positive for CBD analytes as both are present to varying degrees in cannabis variants. Positivity rates by IA were similar to previously published results. It is concluded that there is widespread use of cannabis and CBD products in this population.

### Hemp, Cannabis, Workplace Drug Testing

## K47 A Comparison of Novel Psychoactive Substances (NPS) Positivity in Postmortem Investigations, 2019–2020

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**Learning Overview:** The goal of this presentation is to provide attendees an overview of the fluctuations and new trends related to NPS in Postmortem (PM) forensic investigations submitted to a large reference laboratory between January 2019 and June 2020, with focus on the most relevant NPS classes: Novel Synthetic Opioids (NSOs), Designer Benzodiazepines (DBZDs), synthetic stimulants, and Synthetic Cannabinoids (SCs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees to be able to identify those NPS emerging in the illicit drug market in the United States during 2019 and the first half of 2020. Additionally, demographic data as well as concentration levels from different PM cases will be provided as a reference for the forensic science community.

NPS have been steadily emerging on the illicit drug scene since 2008. Illicit manufacturing of different classes of NPS and the alarming increase of NPS-related overdoses over the past decade have raised the need of investing efforts to constantly monitor their appearance, elucidate pharmacological aspects, and implement drug-control policies. Changes in trends in NPS are difficult to predict, and they usually are subject to demographic, economic, and social aspects. For instance, it is expected that the international and national restrictions due to the global coronavirus disease pandemic have had an impact on NPS market flows.

Tracking NPS occurrence, mapping their prevalence, and developing methods using advanced mass spectrometry-based techniques have been essential components of the work in the laboratory to understand NPS use. The laboratory has addressed this problem by expanding its screening libraries to include those novel compounds emerging in the drug market and monitoring their prevalence with subsequent development and validation of confirmatory analytical methodologies. In this study, a comparative analysis between a six-month period January–June 2019 and 2020 was performed focused on NPS-related PM cases. A total of 2,293 NPS-positive identifications within the DBZDs, NSOs, SCs, and synthetic stimulants classes were confirmed in 1,931 PM blood samples. In the first half of 2019, 810 NPS were confirmed in 637 PM blood samples against 1,483 NPS in 1,294 PM blood samples analyzed in the same period in 2020. Interestingly, the positivity of DBZDs was higher than that for NSOs (61% vs. 23%), while in 2019, NSOs surpassed DBZD positivity by almost the same difference (62% vs. 21%). In 2020, within the cases associated to DBZDs, flualprazolam accounted for 58%; it surpassed etizolam (33%), which had been the most frequent finding within this class for the past four years. Furthermore, flualprazolam positivity increased near three-fold by May compared to February, where the highest % increase (relative to 2019) was reached by DBZDs (607%).

As with DBZDs, % increase of synthetic stimulants remained positive over the six months in 2020, with the highest % increase in April (430%) and the lowest in January (22%); eutylone accounted for 84% of these cases followed by alpha-PHP/alpha-PiHP (11%). Within the SCs, 4F-MDMB-BINACA and 5F-MDMB-PICA remained the most prevalent within the scope of testing, although a steady decrease was observed from January to June 2020 with a 116% increase and -52% decrease, respectively.

NSOs, which had been the most prevalent NPS class in recent years, has declined in total positivity confirmations in 2020. Also, while NSO confirmations (excluding acetyl fentanyl) doubled between April and May in 2019, the opposite was observed in 2020; the largest difference was observed in May with a % decrease of -69. Carfentanil has had a steady prevalence in the illicit drug market since its emergence in 2016 and, within the NSO class, carfentanil has been the most abundant finding in 2020 (29%). U-47700, 3-methylfentanyl, 2-furanylfentanyl, and para-fluoroisobutyrylfentanyl, first identified between 2016–2017, are still involved at low frequencies in PM cases. Additionally, two new NSOs, differing from the typical 4-anilidopiperidine core of the fentologs, isotonitazene and brophine, emerged in 2019 and 2020, respectively. Furthermore, isotonitazene accounted for 30% of positive NSO-involved PM investigations in the first half of 2020.

The factors affecting the observed fluctuations in NPS use in both periods (2019 and 2020) remains unclear, however, the data shown in this study reveal that not only are “old” NPS are still around, but also that this phenomenon keeps evolving toward the use of increasing breadth and variety of substances, with an increase in DBZDs and synthetic stimulant use, and the emergence of non-fentanyl -elated opioids.

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### Novel Psychoactive Substances, Postmortem, Forensic



## K48 A Flualprazolam Study on Postmortem Samples

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**Learning Overview:** The goal of this presentation is to provide additional reference data on flualprazolam, a new synthetic benzodiazepine, in postmortem blood, urine, and tissues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that the importance of evaluating flualprazolam on collected postmortem samples may provide an understanding in the distribution of this benzodiazepine in multiple postmortem matrices.

Benzodiazepines are commonly used for their sedative effects by increasing the level of the inhibitory neurotransmitter Gamma-Aminobutyric Acid (GABA) in the brain. These sedative effects are important to consider when interpreting postmortem cases. In recent years, many Novel Psychoactive Substances (NPS) have become popular, particularly flualprazolam for NPS benzodiazepines. Flualprazolam has a similar structure and presumed similar effects as alprazolam. Due to the lack of analysis and quantification of flualprazolam in many toxicology laboratories until recently, not many postmortem reference concentrations can be currently found. At the Orange County Crime Lab in California, the drug prevalence of flualprazolam drastically increased in the past three years. Postmortem heart blood, vitreous humor, liver homogenate, brain homogenate, gastric contents homogenate, and urine samples were selected to be quantitated after positive results from screening in the heart blood. A validated quantification method for flualprazolam from 4–256ng/mL by DPX WAX-S tips on a liquid chromatography/tandem mass spectrometry was used to analyze all samples. The method validation followed the Scientific Working Group for Forensic Toxicology (SWGTOX) and American National Standards Institute/Academy Standards Board (ANSI/ASB) method validation documents, including calibration model, stability, ion suppression/enhancement, bias and precision, limit of detection, limit of quantification, and dilution integrity. A total of 36 central blood samples were analyzed with 22 samples having a flualprazolam concentration between 4.24–48.03ng/mL, with an average of 16.33ng/mL and a median of 9.95ng/mL. Of those 36 cases, not all tissues could be acquired. The total number and average for each sample type is: urine—21 samples with an average of 14.36ng/mL un-conjugated flualprazolam; vitreous humor—22 samples with an average of 7.70ng/mL; brain homogenate—23 samples with an average of 23.23ng/g; liver homogenate—23 samples with an average of 50.71ng/g; and 22 samples of gastric contents homogenate with an average of 0.33mg.

From this study, the flualprazolam concentrations in the central blood and tissues appear lower than those seen in cases containing alprazolam. Flualprazolam does not appear to absorb into vitreous humor well as it was only present at detectable levels in 5 of the 22 cases. Of the 34 cases where a cause of death had already been decided by the medical examiner, 13 contained flualprazolam as a contributing factor. A continuation of concentration must be collected and reported. The availability of multiple matrices will aid in the understanding of drug distribution within the body.

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### **Flualprazolam, Benzodiazepine, Postmortem**

## K49 The Detection of Kavain in Powder: Death Scene Evidence and Postmortem Blood Analysis

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**Learning Overview:** The goal of this presentation is for attendees to accrue insight into the analytical identification of kavain in a non-biological evidence sample recovered from a death scene and in the case-corresponding postmortem blood obtained at autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underscoring the importance of strong collaborative working relationships between medical examiners and toxicology laboratories in facilitating death investigations that suspect novel or non-routine chemical compounds.

**Case History and Circumstances:** A 56-year-old male was found dead in his secure bedroom by his housemate, who also reported a history of alcoholism with recent detoxification program participation. The decedent's former spouse reported his experimentation with kava and kratom. The scene investigation revealed bloodstains in the bathroom leading into a disheveled master bedroom. The decedent was nude and collapsed next to the bed. Multiple envelopes of a yellow powdery substance were discovered on several room surfaces; likewise, numerous similar plastic bags were located in bedroom dresser drawers. Several old prescription medications were found to include: gabapentin, temazepam, clonazepam, lisinopril, mirtazapine, and sildenafil. A ceramic soap holder in the bathtub was broken, noting sharply edged pieces in the wet bathtub with a bloody towel. Non-velocity blood spatters and smears were noted on the bathroom and bedroom floors and walls.

**Methods:** Autopsy specimens submitted for toxicological analyses included iliac blood, urine, and vitreous humor; a tan-colored powder in a plastic bag recovered from the scene was submitted for non-biological drug identification analysis. The toxicology analyses entailed Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) for volatile compounds and a comprehensive drug screen in blood following organic extraction and analysis by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). A urine drug screen for 28 drugs/drug metabolites was conducted following enzymatic hydrolysis and Supported Liquid Extraction (SLE) by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). Confirmatory quantitative analyses for gabapentin (gabapentin d<sub>10</sub> as IS, Lower Limit of Quantification [LLOQ] 0.5mcg/mL) and mirtazapine (mirtazapine d<sub>3</sub> as IS, LLOQ 2.5ng/mL) were achieved by UPLC-MS/MS. For the analyses of kava in the non-biological powder and blood, the laboratory adopted a three step strategy: (1) identification and procurement of available certified reference standards for compounds in the kavalactone class, (2) analysis of the non-biological powder for the kavalactone compounds, and (3) analysis of the postmortem blood specimen for the compound(s) detected in the non-biological powder. Kavalactone compounds monitored by UPLC-MS/MS were kavain, methysticin, and yangonin. All kavalactone analyses were completed qualitatively.

**Results:** The non-biological powder was positive for the presence of kavain. Gabapentin (3.3mcg/mL), mirtazapine (96.9ng/mL), and kavain (qualitative) were detected in iliac blood. Mitragynine was presumptively identified by LC/qTOF/MS and quantitatively confirmed (180ng/mL) by a reference laboratory. Urine revealed the qualitatively positive result of 7-aminoclonazepam. The cause of death was mixed drug toxicity with kavain, mitragynine, gabapentin, and mirtazapine. The manner of death was accident.

**Discussion:** In the United States, kavain is available without a prescription as a dietary supplement, while in Europe it is prescribed as an antidepressant or muscle relaxant. The psychoactive effects may include mild sedation or stimulation and euphoria. Mitragynine demonstrates stimulant properties at low dose; however, at high dose, psychosis, hallucination, delusion, and confusion may occur.

**Conclusion:** The decedent's mitragynine blood concentration is consistent with reports of fatal cases wherein it is detected with at least one other drug. Although the concentrations of gabapentin and mirtazapine appear benign, the combination of kavain and mitragynine may potentiate lethal central nervous system toxicities.

### Kavain, Toxicology, Blood

## K50 Over the Counter (OTC) Drugs Encountered in a Large Population of Postmortem Pediatric Cases

Jennifer L. Turri Swatek, MS\*, Newnan, GA 30265; Michael E. Lamb, MSFS, NMS Labs, Horsham, PA 19044

**Learning Overview:** After attending this presentation, attendees will have insight into pediatric exposure to OTC medications in the postmortem population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that commonly available medications are susceptible to misuse, and special consideration needs to be given when assessing toxicological findings in the pediatric population.

In forensic toxicology, the pediatric population requires special focus when evaluating positive findings. In particular, administration of OTC drugs needs careful consideration because of the many toxicokinetic and toxicodynamic differences (e.g., metabolic capabilities, body size, etc.) between pediatric and adult populations. Due to these differences, dosages given to the pediatric population (0 days–19 years), particularly those less than five years of age, tend to be lower than that of an older population. This presentation provides insight into pediatric exposure to OTC medications in the postmortem population.

Postmortem pediatric data from a nine-and-one-half-year period (January 2010–July 2020) were compiled. A total of 2,123 positive cases contained one or more of the following common OTC medications: antihistamines (brompheniramine, chlorpheniramine, diphenhydramine, doxylamine, and pheniramine); pain relievers (acetaminophen, naproxen, ibuprofen, and salicylate); cold/flu medications (dextro/levomethorphan, guaifenesin, ephedrine, and pseudoephedrine); Gastrointestinal (GI) aids (dicyclomine and loperamide); and sleep aids (melatonin).

	Antihistamines	Pain Relievers	Cold/Flu Medications	GI Aids	Sleep Aids
Neonate (N); ≤28 Days	37	26	15	1	0
Infant (I); 29 days - <1 year	93	115	57	3	4
Toddler (TD); 1 - 3 years	126	122	45	6	5
Pre-School (PS); 4 - 5 years	25	26	17	0	2
School Age (SA); 6 - 12 years	72	63	38	4	2
Teens (TN); 13 - 19 years	521	294	286	18	0
<b>Totals</b>	<b>874</b>	<b>646</b>	<b>458</b>	<b>32</b>	<b>13</b>

**Table 1:** Number of positive cases of common OTC medications in the postmortem pediatric population.

Antihistamines, cold/flu medications, and pain relievers are the most common classes of drugs encountered in the postmortem pediatric population. When considering the data, it must be noted that many of these drugs may be co-administered in the form of one medication containing multiple drugs or multiple drugs being used in conjunction with one another. In addition, some drugs may have a variety of uses (e.g., antihistamines used as sleep aids). Of note, a trend can be seen where the infant and toddler age groups have a higher number of positive cases, followed by a dip, then a large increase in the teen population. These infant and toddler populations represent a group that relies on medication administration from a more mature figure. As dosages are often lower for the younger population, incorrect handling of medications by the individual administering the drug(s) may result in unintentional overdoses. The older, teenaged population represents a group that is capable of self-administering OTC medication. With the widespread availability of OTC medications, intentional recreational abuse (e.g., dextromethorphan and loperamide) and suicidal overdoses are more prevalent in this population. Case examples demonstrating use of OTC medications in the pediatric population will be discussed.

Overall, this presentation demonstrates that commonly available medications are susceptible to misuse, and special consideration needs to be given when assessing toxicological findings in the pediatric population.

### Pediatric, OTC Drugs, Postmortem

## K51 Bladder Wash: A (Not-So) Alternative Specimen for Postmortem Forensic Toxicology

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**Learning Overview:** The goal of this presentation is to determine the potential of bladder washes (BW) as an alternative specimen in postmortem toxicology in cases where there is no urine available to analyze.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the efficiency of BW drug analyses compared to traditional blood and urine drug analyses. The findings in this study may influence postmortem toxicology laboratories to implement BW as a possible alternative specimen.

Urine is an excellent sample in postmortem forensic toxicology to provide evidence of previous drug use. However, there are many cases in which the bladder is voided prior to their autopsy or dehydration occurs postmortem. In these cases, it is possible to wash the bladder with distilled water or saline and thus collect the BW and any available residual urine for drug screening and confirmation. The San Francisco Office of the Chief Medical Examiner (OCME) has made the collection of BWs at autopsy an option when urine is not available. While BWs are not conventional, this study aims to determine its use in postmortem forensic toxicology.

Data from analysis of BW samples collected at the OCME were analyzed to assess the efficiency of this alternative sample in comparison to blood from the same individual by determining the identities of individual analytes and their metabolites ( $n=100$ ). The BW samples, which were obtained by rinsing the bladder wall with 10mL of 0.9 sodium chloride solution and then collecting the resulting solution via a syringe, and the blood samples (peripheral and/or cardiac) were analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Authentic case results following routine testing showed that for individual analytes, there is an 80%–90% correlation between blood and BW results. In addition, when examining both parent drug and metabolite data, this estimate correlation increases. The results have also shed light on noteworthy findings regarding specific analytes. For example, in the preliminary study, it was found that in two out of the seven fentanyl/norfentanyl cases, both drugs were detected in the BW, but not in the blood. Three other fentanyl cases had both drugs detected in the blood, with either one or neither of the drugs detected in the BW. Also, the preliminary study showed that the primary metabolite of heroin, 6-Acetylmorphine (6-AM) was BW analyses, BW and urine drug analyses from corresponding postmortem cases were obtained to create a control cohort ( $n=40$ ). To assess the current knowledge and opinions regarding BWs in postmortem toxicology, a survey was sent out to various coroner and medical examiner offices across the country. As of now, the results of the survey are pending.

This preliminary study appears to indicate that standardizing the collection and analysis of BWs in postmortem toxicology will provide forensic pathologists with a comprehensive toxicological profile in cases where urine and/or other biological specimens are not available for collection and subsequent analysis. In addition, since two different specimens from the same case can be used for a drug confirmation, analyzing a BW in addition to a blood sample can allow a forensic toxicologist to confirm drug results without requiring traditional screen and confirmation regimes.

### Bladder Wash, Alternative Specimen, Postmortem Toxicology

## K52 Abuse Deterrent Formulation (ADF) Oxycodone: A Ten-Year Study of Driving Under the Influence of Drugs (DUID) and Postmortem (PM) Oxycodone/Oxymorphone Blood Trends

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**Learning Overview:** After attending this presentation, attendees will be familiar with the formulation change to oxycodone tablets in 2010 and oxymorphone in 2012 and what impact(s), if any, the changes have had on oxycodone and oxymorphone blood concentrations in DUID and PM toxicology casework in the decade that followed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying information regarding oxycodone/oxymorphone blood concentrations and trends in DUID and PM populations following the drug tablet reformulation of oxycodone in 2010 and oxymorphone in 2012.

With the intent of deterring opioid abuse in the early 2000s, the pharmaceutical industry focused on developing ADFs for its products. In August 2010, the Food and Drug Administration (FDA) approved the first ADF product, commercial oxycodone tablets, which was soon followed by ADF oxymorphone in 2012. Both formulations made the tablets harder and more difficult to manipulate, crush, snort, and/or inject. Now more than ten years later, an FDA advisory committee recently concluded that while ADF oxycodone did reduce abuse by non-oral routes, the reformulation did not produce a meaningful reduction in overall opioid abuse, overdose, and death. The purpose of this work is to describe the trends of oxycodone and oxymorphone use in DUID and PM populations in the decade following oxycodone's reformulation.

Oxycodone- and/or oxymorphone-positive cases received at NMS Labs between January 2010 and July 2020 were reviewed. A total of more than 43,600 oxycodone and 16,000 oxymorphone cases were reported across all matrices, representing overall case positivity rates of approximately 4.7% and 1.7%, respectively. Cases were screened via immunoassay, Gas Chromatography/Mass Spectrometry (GC/MS), or Time-Of-Flight/Liquid Chromatography/Mass Spectrometry (TOF/LC/MS) depending on case type, matrix submitted, and year of analysis. For ease of discussion, this presentation will focus primarily on blood analysis results. Blood confirmation testing was performed via Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) with reporting limits of 5.0ng/mL for oxycodone and 1.0ng/mL for oxymorphone.

Biennial snapshots of average free analyte concentration are reported below in Tables 1 (DUID blood) and 2 (PM blood—all types). Both case types show an approximate 2x decrease in average oxycodone blood concentrations and 6–7x decrease in oxymorphone blood concentrations over the ten-year time range. Following reformulation of oxycodone in 2010, DUID casework trends indicate a steady six-year decrease in average oxycodone blood concentrations before plateauing near 60ng/mL. In contrast, DUID oxymorphone concentrations saw a sharp drop of nearly 75% in average blood concentration after the 2012 reformulation. Furthermore, average PM blood concentrations of both oxycodone and oxymorphone abruptly dropped by 55%–60% in the first two years following reformulation of their respective tablets before plateauing. This data supports the idea that ADF reformulations reduced some but not all routes of oxycodone/oxymorphone abuse.

Table 1 – DUID Blood		2010	2012	2014	2016	2018	2020 (Jan-July)
Oxycodone – free (ng/mL)	N	120	860	970	1800	730	240
	Average (±SD)	120 (± 130)	100 (± 120)	90 (± 120)	70 (± 100)	60 (± 90)	50 (± 80)
Oxymorphone - free(ng/mL)	N	10	70	330	770	240	60
	Average (±SD)	10 (± 10)	20 (± 10)	5.0 (± 7.0)	7.0 (± 60)	4.0 (± 6.0)	2.0 (± 3.0)

Table 2 – PM Blood (all)		2010	2012	2014	2016	2018	2020 (Jan-July)
Oxycodone - free (ng/mL)	N	2600	3700	4000	5100	5000	2800
	Average (±SD)	810 (± 9200)	460 (± 2300)	400 (± 1700)	450 (± 4100)	310 (± 1300)	340 (± 3600)
Oxymorphone - free (ng/mL)	N	810	1100	2300	3500	3200	1600
	Average (±SD)	100 (± 610)	70 (± 230)	40 (± 240)	20 (± 90)	10 (± 90)	10 (± 70)

**Note:** Average ± SD values have been truncated to simplify reporting.

From this data, oxycodone/oxymorphone prevalence appears to be declining with maximum DUID and PM positive reports being issued in 2016 and 2017, respectively. While this is good news, prevalence and concentrations cannot be the sole indicators for measuring abuse. Additional explanations for oxycodone decline include restrictions and/or hesitations by medical professionals to prescribe opioids and the rise of abuse in both heroin and synthetic opioids. Given this information, it is difficult to attribute the impact that ADF reformulations alone have had on opioid use and abuse over the past ten years.

### ADF Oxycodone, Oxycodone, Opioid Abuse

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## K53 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 21<sup>st</sup> Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation of issue-specific concern, including pharmacokinetic data and other relevant ancillary information, followed by attendee 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.

Due to the unusual circumstances of this year's meeting, the presentations this year will draw from the annals of cases at NMS Labs. Four cases of toxicological findings in postmortem pediatric cases will be presented. The first case involves the death of a newborn that was born at home and never received afterbirth care. Death occurred within days with toxicological findings that included heroin products and cocaine. Questions regarding *in utero* versus neonatal exposure needed to be addressed. The perimortem facts are unusual and complicated the ability to interpret the findings.

Dr. Michael Rieders will speak to the developing relationship between the emerging mix of toxic adulterant cutting agents in drugs of abuse and the rise in maternal and fetal morbidity and mortality. Over 20,000 pregnant substance use-dependent women expose themselves and their developing fetus to myriad toxic adulterant cutting agents during pregnancy. Umbilical cord tissue from the neonate may reveal these substances, allowing an appropriate treatment plan to be developed as well as assisting medical examiners in cases of stillborns.

Dr. Laura Labay will address a case of a 13-month-old who was found unresponsive in a shared room. The infant was extremely warm to the touch. Resuscitative efforts were unsuccessful, and the infant was pronounced dead. Analysis showed a melatonin result of 210ng/mL in blood. Melatonin is an endogenous hormone that regulates sleep patterns. It is available in varying formulations and dosages and is marketed as a natural substance that can alleviate insomnia. Melatonin has been administered without appropriate authorization in daycare settings and by care providers attempting to induce sleep.

Ms. Jenni Turri Swatek will address two cases in which over-the-counter cocktails of cold-related preparations were administered to young children. She will highlight the dangers of casual use of these substances for misguided purposes and fatal outcomes.

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### Pediatric, Toxicology, Postmortem

## LW1 China and the History of Fingerprinting: A Forgotten Chapter?

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**Learning Overview:** After attending this presentation, attendees will better understand how officials in premodern China used fingerprints to make identifications, how these practices differed from modern fingerprinting, and how the patterning of friction ridge skin has been described and classified in different places and historical periods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving upon commonly accepted narratives of the history of fingerprinting with new facts and interpretations and by promoting a deeper understanding of the history, development, and present-day circumstances of this field.

Textbooks and other writings on fingerprinting often contain brief discussions of the history of this field. Such discussions generally mention the fact that historical precedents for fingerprint identification can be found in Chinese history. The *Fingerprint Sourcebook* of the National Institute of Justice, for example, notes that “[the] Chinese were the first culture known to have used friction ridge impressions as a means of identification.”<sup>1</sup> Another example: “Chinese dynastic history is filled with examples of fingerprints used as personal marks long before European historical fingerprint records.”<sup>2</sup> Ever since Francis Galton’s (1822–1911) *Finger Prints* (1892), those who have written on the history of fingerprinting have marshaled a range of evidence—finger impressions on ancient Chinese pottery, deeds and contracts with inked fingerprints, and scattered textual references—to describe the role that China played in this history.<sup>3</sup>

There is, in fact, a premodern Chinese usage of fingerprints that has been largely ignored in English-language histories, yet that is arguably the application that was closest to modern fingerprint identification. During the last imperial dynasty, the Qing (1644–1911), Chinese officials were required by law to examine convicts’ friction ridge patterning in specific ways. Officials were to record the configuration of two different pattern-types—known as *dou* and *ji*—across the ten fingers. The Chinese word *dou* was used to refer to fingerprints that were circular or spiral-shaped, similar to the “whorls” of today. The word *ji* was used for fingerprints that were asymmetrical or crooked in form. When examining a convict’s ten fingers, officials might record, for example, that there were *dou* patterns on this and that finger and *ji* patterns on others. Given that the specific configuration of *dou* and *ji* on the hands could be expected to vary across different individuals, this method provided a simple way of verifying a convict’s identity, used in conjunction with additional identifying information such as scars or tattoos.

Long before the late 19<sup>th</sup>-century innovations of Edward R. Henry (1850–1931), Juan Vucetich (1858–1925), and others, which made it possible to efficiently organize large numbers of paper-based fingerprint records, this older Chinese system provided a way for officials to describe the full profile of a person’s ten fingerprints in systematic ways and to use this information in identification work. Given the utility of this method, it should not be surprising that Chinese officials continued to use it well into the 20<sup>th</sup> century, during a period when modern techniques of fingerprint identification were also being introduced.

After explaining how this identification method worked and briefly comparing it to modern ways of classifying, registering, and matching fingerprints, this presentation will conclude with a discussion of the modern afterlife of this older system. In the 1910s and 1920s, those who translated modern knowledge of fingerprinting into Chinese (including during and after Vucetich’s visit there in 1913) used the older terminology of *dou* and *ji* as the basis for the modern Chinese words for “whorl” and “loop,” terms that are still used today.

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### Fingerprints, Identification, China

## LW2 Pursuing Injustice Through Truth in Conjecture: The Hunt for a Scapegoat During an Ancient Pandemic

Matteo Borrini, PhD\*, Liverpool John Moores University, Liverpool, AE L3 3AF, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of pursuing justice through scientific evidence without any cultural or social bias.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by analyzing a historical trial as an illustration of the importance of scientific evidence-based investigation and prosecution, significant especially when social factors can influence the case.

Between 1629 and 1631, a massive outbreak of bubonic plague struck the north of Italy, in particular Milan and the neighboring cities. Alessandro Manzoni described the social impact of this pandemic in the historical novel, *The Betrothed (I promessi sposi)* in 1827.<sup>1</sup> During the preparation of his book, the author collected data that he published later in the monographic essay, *History of the Infamous Column* (*Storia della Colonna Infame*).<sup>2</sup> In this paper, Manzoni recounts the trial against two alleged plague-spreaders, held responsible for the pestilential contagion through mysterious substances.

On June 21, 1630, the commoner Caterina Rosa spotted the health commissioner, Guglielmo Piazza, going close to the buildings' doors and walls in Vetra Street. She believed that he was anointing the walls with a dark substance to spread the plague. That accusation was later confirmed by one of her neighbors, Ottavia Bono.

The police misinterpreted some ink stains on the walls as the pestilential ointment and arrested the suspect "smearer" (*untore*), whose hands appeared stained. Piazza, subjected to interrogation and torture, denied the accusation. Only under the pressure of the inquisitors, who promised him impunity and freedom, did Piazza confess to having received the pestilential ointment from Giangiacomo Mora, a local barber who also sold balms and potions that he claimed cured disease.

An inspection of Mora's house was conducted only after his arrest, and the investigators discovered traces of a mysterious unguent. Even if Mora explained the harmless nature of the preparation, which was a mixture of ash and boiling water used to wash clothes, the judges and experts appointed by the court identified the balm as a pestilential potion capable of spreading the plague.

The trial continued, based on the use of torture rather than an actual search for evidence. The violent methods led the two accused to contradict their statements several times and finally confess the non-existent guilt to end the atrocious physical and psychological tortures. They also accused five alleged accomplices in a desperate attempt to save their lives, having faith in the investigators' groundless pledges.

Finally, the court decreed the death sentence for all the unjustly accused, and the execution took place on August 2 by the breaking wheel after parading the accused through the city' districts, where they had their "guilty" hands amputated. Mora's house-shop, allegedly the place where the poisonous ointment had been produced, was demolished. A pillar, known as the "Infamous Column," was erected in that location as an enduring memory of the punishment incurred by those spreading the plague.

The monument was only removed in 1778 and recognized as a testimony of infamy no longer charged to Mora but to those who had committed a colossal injustice.

Alessandro Manzoni intended to underline the mistake committed by the judges and the abuse of their power, which trampled every form of human common sense, driven by an utterly unfounded conviction and by a fear linked to the terrible environment caused by the epidemic.

Under a historical and forensic perspective, it is essential to understand how the pandemic killed approximately half of the Milanese population and put the people under immense stress, already scared by an unknown and merciless disease. The fear fed the diffusion of popular superstitions that were used in an attempt to rationalize the mysterious disease. Ordinary people were identified as responsible, usually picking them from ethnic and other minorities; at other times, supernatural forces were invoked. One of them is the *nachzerer*, the shroud-eating vampire who spread the plague from the grave through demonic action.

The goal of this research is to describe the risk of an unfair trial when the investigation and judgment are influenced by social environments, stressful factors, media, and public opinion. This presentation also stresses the importance of a trial based on scientific evidence and the reliability of investigations sustained by scientific research, training, and forensic standards.

### Reference(s):

1. Alessandro Manzoni. *The Betrothed (I promessi sposi)*. 1827.
2. Alessandro Manzoni. *History of the Infamous Column (Storia della Colonna Infame)*. 1840.

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### Trial, Pandemic, Evidence-Based Investigation



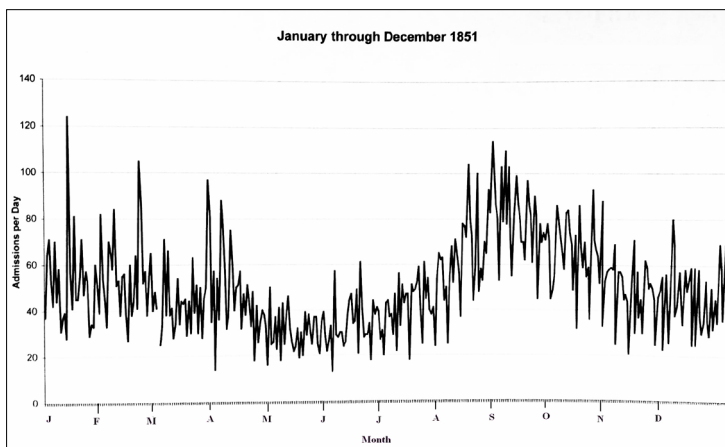
## LW3 19<sup>th</sup>-Century Charity Hospital Admission Records—A Window Into Irish Life and Times

Colleen M. Fitzpatrick, PhD\*, Fountain Valley, CA 92708

**Learning Overview:** After attending this presentation, attendees will understand the nexus between historical records and modern forensic analysis, as illustrated by 19<sup>th</sup>-century public hospital admission registers. This presentation will illustrate how much about the “life and times” can be derived from what 150 years ago was considered routine record keeping, when names, ages, and other data were recorded as patients were admitted to the Charity Hospital in New Orleans, LA. Each individual record is valuable as perhaps the only remaining glimpse of a life that otherwise would have been forgotten over a century later. Yet taken as a whole, the tens of thousands of records that eventually filled the admission books can be viewed as a collection of data rich with information about 19<sup>th</sup>-century economics, politics, and living conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how much insight into the past can be developed by applying modern forensic techniques to routine recordkeeping from so long ago. While it is exciting to see modern forensic tools applied to modern cases, it can be even more interesting to see what hidden information these same tools can extract in a context inconceivable to those who lived so many generations in the past.

In 1850, New Orleans was a city with a population of about 116,000. In the next few years, however, the town experienced a tidal wave of a quarter-million immigrants, over half of whom were Irish escaping the potato famine ravaging their country. Starving and destitute, the refugees offered a ready source of cheap labor. As pressure mounted on the city to drain land to accommodate its dramatic increase in population, the fair-skinned Irish were put to work in the subtropical sun, often standing knee deep in stagnant pools of mosquito-infested water. Disease was rampant and the free medical care offered by the Charity Hospital was the only alternative available to New Orleans’ poorest of the poor.



The admission data from this era are revealing. The number of admissions for 1851 was typical, drifting up and down, rising to a maximum in August, and then tapering off at the end of the year. There are positive and negative spikes superimposed on the curve. The largest positive spikes occur on specific days when the number of admissions suddenly increase well above the average, then quickly die away within a day or so. The negative spikes seem to occur on a more regular basis. These increases and decreases were caused by something that caused people to get sick—especially the Irish, who accounted for the majority of the hospital’s intake.

The answer could lie at least partially with the weather, which is normally hot and humid during the summer in New Orleans. But if so, why did the weather affect the Irish more than others, and what caused the three-week lag between the peak of the rainfall and the maximum in admissions? What caused the one-day spikes? How can the admission records for 1851 and those of other years be superimposed on a background of immigration and Irish culture?

### Hospital, Epidemic, Irish

## LW4 “The Man Who Never Was”—Was He the Man the British Say He Was?

*Pete Speth, MD\*, Forensic Consultations, Wenonah, NJ 08090*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of why an unmet scientific review is needed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating challenges for forensic pathologists and taphonomists, physics 101, and questions of war ethics.

**Operation Mincemeat:** Tens of thousands of military lives were spared during the allied invasion into the belly of Europe through Sicily. This was accomplished by deceiving Hitler with false documents into believing the invasion would be through Sardinia and Greece. He moved many forces out of Sicily.

A fully uniformed corpse made up as a fictitious “Major Martin,” who was to be believed to be carrying top secret documents in a briefcase to generals in North Africa, but who drowned during a plane crash into the Sea of Cardiz. Actually, the corpse was delivered by submarine. It washed ashore near Huelva, Spain, and was autopsied by a medical examiner who determined cause of death as drowning in the 60° seawater five to six days earlier. The autopsy report burned in 1976.

The corpse is said to have been a young adult London vagrant, Glyndwr Michael, who suicidally took yellow phosphorus and died in the hospital two days later on January 23, 1943, after failed life-saving efforts. The coroner wrongfully determined Glyndwr as having no next-of-kin and falsely certified the body as shipped out of the country. The corpse was then kept in a morgue refrigerator for 73 days, followed by another 14 days in a morgue freezer.

A double-hulled, 74-inch long, 22-gauge steel welded cylinder was manufactured; its outer diameter was 22" and its inner diameter was 19", with 1.5" asbestos wool insulation between the two hulls. A 22-gauge plate was welded over one end and a 22-gauge lid bolted shut over the other against a grommet. The cylinder proved to be airtight.

On April 17, the corpse was taken out of the freezer. The cylinder was placed upright, open end up. Twenty-two pounds of dry ice were placed in it to cool and to remove oxygen. The corpse's ankles were thawed to put on boots. A “Mae West” life jacket was placed around the chest, and the briefcase for false documents was chained to the wrist. A blanket was wrapped around the corpse, taped to protect it. The corpse was placed into the cylinder, “packed” with dry ice, and bolted shut.

Strangely, the submarine sailed around from Blythe to Firth of Clyde, and the cylinder containing the corpse was driven 430 miles through darkness to the Firth. After loading the cylinder into the submarine, departure was on April 19. The cylinder was on a torpedo rack where the sailors slept; they were told it contained optics. Arrival was about one mile from the beach on April 30. The cylinder was brought onto deck. Five officers were “in the know.” The cylinder was opened, the corpse removed, the blanket unwrapped, the Mae West inflated, and a short prayer offered. The cylinder was dumped overboard and was found five hours later by a fisherman; it was carried by donkey to a ferry, sent upriver to Huelva, and autopsied the next day. The body was buried in Huelva by the British as “Major Martin,” eventually attributed to Glyndwr Michael.

**Questions:** What was the degree of decomposition at autopsy? Does it comport with five to six days in 60° seawater? What about the CO<sub>2</sub> sublimation pressure in the airtight cylinder? Should it have violently discharged upon unbolting? Should not the stench of decomposition have been noticed by sailors when officers returned below? Was the Firth of Clyde chosen because of the 379 sailors who drowned there on March 27, when the aircraft carrier HMS Dasher exploded? Sir Erin Montagu, who conceived of and managed Operation Mincemeat, met with the submarine captain three days later. The wife of one of the drowned sailors, John Melville, was singularly denied the viewing of his body and buried him in the family cemetery. In 2004, Lieutenant Commander Mark Hill held a memorial tribute in the Mediterranean in honor of John Melville as the “man who never was.” Not long after, the Ministry of Defense denied this. Who is buried in Huelva? Glyndwr Michael's family eventually were found and honored. Mrs. Melville never found her husband.

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### Decomposition, Sublimation, Ethics

## Y1 A Bone Elemental Age-at-Death Estimation of Deceased Adult Females: A Pilot Study

Melissa Bernard, MSc\*, Longueuil, PQ J4L1J4, CANADA; Tracy Rogers, PhD, University of Toronto at Mississauga, Mississauga, ON L5L 1C6, CANADA

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of bone elemental analysis using a scanning electron microscope with energy dispersive X-rays.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering data on Calcium/Phosphorus (Ca/P) ratios in adult females as a potential alternative to the common age-at-death estimation method.

In forensic anthropology, common age-at-death estimation methods rely on macromorphology of the skeleton, such as dental development and long bone length for juvenile individuals and teeth or bone wear for adult individuals. Biological and environmental factors such as health, biomechanical stress, socioeconomic status, and sex are factors among others that can affect bone aging and the appearance of wear in adult individuals. To mitigate these biological variations between individuals, common macromorphological methods provide a wide range of estimated age at death that can span 15–20 years or more for the elderly population. Developing a more precise age-at-death estimation method could help reduce those age ranges in adult individuals and provide a more precise biological profile to better support death investigations.

Studies showed that in healthy individuals, the inorganic osseous component consists primarily of Ca and P with a high molar ratio during fetal development and skeletal growth that gradually declines to a plateau at a ratio of 1.67 when reaching skeletal maturity.<sup>1</sup> It was hypothesized that natural aging processes impact levels of sex hormones, causing osseous Ca levels to decrease and Ca/P ratios to decline in the elderly population.<sup>2</sup> A pilot study on nine adult males demonstrated a linear relationship between declining Ca/P ratios and age at death at the midshaft of the femur.<sup>3</sup>

To control for sexual variation in bone aging and provide missing data on Ca/P ratios, the present pilot study investigated Ca/P ratios in relation to age at death of 16 adult females aged 49 to 101 years. The data collection was conducted blind to age at death, controlling against individuals with known osteoporosis or bone cancer diagnosis. Medallions were harvested from the femoral midshaft on adult females previously preserved for medical teaching purposes. The femoral cross-section was observed and analyzed using a scanning electron microscope with an energy dispersive X-rays. Ten fields of view of secondary osteons per individual were selected and analyzed elementally using backscatter electrons. The Ca/P ratios were calculated for each field of view, which was then averaged per individuals. The resulting 16 averaged Ca/P ratios were analyzed in relation to known age at death, with and without outliers, to identify possible disparities that could be due to biological variations within and between individuals.

The results of this pilot study were inconclusive; another study with a larger sample size of individuals representing all adult age categories for both male and females is necessary before evaluating if Ca/P ratio is a viable alternative method for age-at-death estimation in adult individuals.

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Age-at-Death Estimation, Elemental Analysis, Calcium/Phosphorus Ratio



# Young Forensic Scientists Forum Posters – 2021

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## Y2 “The Squeakiest Wheel Doesn’t Always Get the Grease”: Indigenous Perceptions on the Highway of Tears Missing and Murdered Indigenous Women and Girls (MMIWG) Investigations

*Steff King, BS\*, Vancouver, BC V5L 5G5, 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 be better informed regarding the concerns and perceptions that Indigenous families and friends of MMIWG have on how investigations are conducted and where further research is needed to address these concerns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by acknowledging the impacts homicide and missing persons investigations can have on victim’s communities, especially when discussing the Canadian Indigenous community. This presentation will also exemplify that continued conversation about investigative practices between scientific professionals, law enforcement, and the victim’s community needs to be an ongoing effort to help mitigate further trauma, even if national recommendations have already been set in place to address existing concerns.

Canada is well-aware of its situation with MMIWG. Yet there is a notable limit to what is public knowledge and what has a level of importance when it comes to MMIWG investigative practices for both prominent Highway of Tears and related Canadian cases. This exploratory study began as a content analysis of news articles, inquiry/commission reports, and academic literature to determine both what information about the Highway of Tears cases are available for public access and how exhaustive that information is. The results uncovered that while there has been an increase in media acknowledgment and government conversation about the Highway of Tears MMIWG cases in particular after the early 2000s, there remains a vacant hole in the prepared narrative of investigative practices. This hole is largely only addressed by the Indigenous community, leaving them to lay the groundwork for identifying where they perceive investigations fall short and creating community organizations to address the situation themselves.

For this research development, a subsequent content analysis of publicly accessible Indigenous testimonies about experiences with MMIWG investigations uncovered common themes in perceptions on how cases were conducted. From such perspectives, flaws in MMIWG investigations were identified as inaccurate victim portrayals in the media, unequal investigative support, slow responsiveness by and distrust in police, withheld case information, and lacking support and assistance from non-Indigenous organizations. Accompanying these themes were occasional accounts of successes in investigative practices, recognition of the difficulties in conducting investigations of such importance, and calls for finding ways to improve the themes identified that negatively affect the Indigenous community.

This research highlights the Indigenous perception of MMIWG investigative practices in Canada as they are the loudest voice in advocating for better practices currently, even though they often have the smallest platform in which to present it to the public, while also arguing to the academic community that further research needs to test these themes in current investigative practices. There is importance in not only determining if these Indigenous concerns have continued in MMIWG investigations following recent inquiries and recommendations, but also if remedies of these concerns have begun and determining how to mitigate the still present and real concerns of Indigenous families and friends of MMIWG victims.

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### MMIWG, Investigative Practices, Indigenous Perspectives

## Y3 Evaluating a DNA Extraction Procedure for Skeletal Remains

Jacob D. Nead\*, James Creek, PA 16657; Christina Neal, MS, Signature Science, Charlottesville, VA 22911; David Russell, MS, Signature Science, Charlottesville, VA 22911

**Learning Overview:** After attending this presentation, attendees will better understand the impact of detergents and proteinase on the efficacy of DNA recovery from skeletal remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results that can be used as proofs of concept to inform laboratories on future steps toward the optimization of bone demineralization buffers and procedures with increasing efficiency.

Skeletal remains are an important source of evidence in a wide variety of forensic cases, including missing persons, mass tragedies, mass graves, and burned victims.<sup>1</sup> The development of an extraction protocol of DNA from skeletal remains would allow a laboratory to expand the range of evidence samples that can be analyzed in-house and thus broaden the range of casework that a laboratory can process. Due to the nature of skeletal evidence, mainly its persistence in harsh environmental conditions, this type of biological evidence often exhibits low-quantity and degraded DNA.<sup>1</sup> The goal of a bone extraction procedure is to recover high-quality DNA while limiting contamination of the sample with exogenous DNA and Polymerase Chain Reaction (PCR) inhibitors.

As part of an internship project, a literature search was conducted to identify the best path forward for the extraction of DNA from bone. Current literature offers a diverse catalog of extraction procedures with varying degrees of efficiency, effectiveness, sensitivity, and downstream application. The proposed procedure includes sample decontamination, total demineralization of the bone powder, concentration of the sample, and purification of the extract.<sup>2</sup> The general workflow of the proposed method is primarily adopted from the Armed Forces DNA Identification Laboratory (AFDIL) procedure, first published in 2007 and more recently expanded upon in a 2019 study.<sup>3,4</sup> Extracts were quantitated using the Investigator® Quantiplex® Pro kit, PCR amplified using the GlobalFiler™ kit, genotyped via the SeqStudio™ genomic analyzer, and analyzed with GeneMapper® ID-X software v1.6.

The goal of this study was to evaluate the efficacy of the proposed method and to consider the optimization of several aspects of the process. Focus was placed on the recovery of nuclear DNA for its diverse downstream applications and high power of discrimination. The method was evaluated based on DNA recovery, consistency of results, inhibition indices, degradation metrics, contamination, and Short Tandem Repeat (STR) profile quality. The effect of adding a detergent to the demineralization reaction was studied by comparing Ethylenediaminetetraacetic Acid (EDTA) buffers with and without a 1% concentration of the detergent, N-laurylsarcosine.<sup>5</sup> Additionally, various volumes of proteinase K spike-in were compared to assess its effect on DNA recovery.<sup>3,4,6</sup> Yield was observed in relation to variable sample input masses.

The method exhibited consistent recovery of DNA from skeletal samples with evidence of high reproducibility and average yields well above the target PCR input for amplification. Inhibition was observed to be negligible; however, the samples did exhibit elevated degradation indices. Degradation was further observed in the STR profiles with signal intensities decreasing from smaller targets to larger loci. The addition of N-laurylsarcosine as a detergent to the demineralization step was generally associated with improved DNA recovery. Similarly, the addition of 200µL of proteinase K was supported as the optimal volume of spike-in at inputs of 100 and 250 milligrams of bone powder. A preliminary trend was observed supporting greater DNA yield with larger sample inputs.

Future studies may investigate increasing DNA recovery to match input requirements for more diverse analyses such as Single Nucleotide Polymorphism (SNP) genotyping and whole genome sequencing. Additional consideration may be given to alternative detergents such as Sodium Dodecyl Sulfate (SDS) to evaluate the impact on the efficiency of DNA recovery. Several recent studies have also provided evidence of potential alternatives to the use of proteinase K as a protein denaturant. Trypsin, another broadly specific protease, has been shown as one alternative.<sup>7</sup> In another publication, the enzyme clostridiopeptidase A exhibited significant reduction in time required to totally demineralize bone samples.<sup>8</sup>

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1. Jon Davoren et al. Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves. *Croatian Medical Journal*. 48: (August 2007): 478-485.
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### DNA Extraction, Skeletal Remains, Evaluation

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# Young Forensic Scientists Forum Posters – 2021

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## **Y4 A Complex Suicide by Drowning and Self-Strangulation**

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**WITHDRAWN**

## Y5 An Unusual Attempted Suicide or a Covered Attempted Homicide? A Neck Stabbing Case Report and Review of the Literature

*Chiara Stassi, MD\*, PROMISE Department, University of Palermo, Palermo 90127, ITALY; Gennaro Baldino, MD\*, Palermo 90100, ITALY; Cristina Mondello, MD, Department BIOMORF, University of Messina, Messina 98123, ITALY; Salvatore Rocuzzo, MD, University of Palermo - Messina, Messina 98124, ITALY; Orazio Malfa, MD, University "Magna Graecia" of Catanzaro, Catanzaro, ITALY; Luigi Cardia, MD, Università degli Studi di Messina, Messina, ITALY; Elvira Ventura Spagnolo, MD, PhD, University of Palermo, Palermo 90127, ITALY*

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**Learning Overview:** After attending this presentation, attendees will better understand what main forensic features are taken into account in order to differentiate a self- from a hetero-inflicted stab wound when only a single person is present.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the role of forensic consultant activity within a clinical setting, especially in those selected cases—such as presence of unusual traumatic wounds—in which a crime report to the Judicial Authority is mandatory.

The distinction between the self- or hetero-infliction nature of stab wounds usually relies on several considerations: the accessibility to the targeted area and the localization of the wounds, being as self-inflicted stabs are localized in sites accessible to the victim's hands (mainly the left chest, reflecting right-handedness, followed by the abdomen and ventral neck), while hetero-inflicted wounds, although most frequently localized on the chest, can be found everywhere on the body, making it sometimes difficult to assume a homicidal act, unless the wounds are localized in clearly inaccessible sites, such as the back. The study of eventual additional wounds and lesions provides further help: the presence of bruises, fractures, or blunt traumas, together with defensive wounds that are randomly oriented and localized on the extensor surface of the hands and forearm or on the back, can offer a homicidal hypothesis; multiple tentative wounds, usually detected close to the lethal one and typically appearing superficial with a parallel orientation and clustering, are indeed indicative of a suicidal stabbing. Further information is provided by the orientation of the stab wound, mostly horizontal if self-inflicted, vertical if hetero-inflicted. As for the state of the victim's clothes, as a general rule those who attempt suicide usually self-stab on exposed skin after removing or pulling up their clothes, thus explaining the absence of any rip; conversely, ripped clothes frequently figure in attempted homicides, with the rips usually overlapping the corresponding stab wounds. Since a strong link is reported between suicide or previous attempts and depressive disorder, the latter occasionally identified by old, linear scars, mainly located on the flexor surface of the wrists, the psychiatric/psychological background of the victim should also be investigated.

All these considerations had been taken into account when a forensic consult was requested following access to the emergency unit of a 52-year-old man with a single stab wound of the neck, who reported self-stabbing in a suicidal attempt. The man was fully conscious, and the vital signs were in range. The wound was penetrative although not lethal, since no vital structure had been involved, and the knife was still onsite, with entry from the right side of the neck and exit from the left side; the blade was obliquely inclined from bottom to top and from front to back. The inclination of the blade was better studied with the aid of a 3D Computed Tomography (CT) reconstruction, showing an inclination from front to back and from bottom to top. Since no other wounds or lesions were present, the clothes weren't available at the time of the consult, and the patient had no history of psychiatric/psychological problems, the stab wound was investigated by a thorough study of its localization, orientation, and inclination of the blade, the possibility of producing a stab wound with the cited characteristics in relation to the range of motion of the wrist and the strength necessary to pass through the neck structures from one side to the other was evaluated.

Based on these considerations, the forensic consultants rejected the self-infliction version provided and agreed on a hetero-inflicted stabbing. According to the Italian Code of Criminal Procedure, both clinicians and forensic consultants submitted a crime report to the Judicial Authority; a criminal prosecution was thus opened, which further confirmed the hetero-infliction nature of the stab wound.

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### Stab Wound, Attempted Suicide, Forensic Investigations



# Young Forensic Scientists Forum Posters – 2021

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## Y6 Racial Bias and Missing Persons Investigations

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**Learning Overview:** After attending this presentation, attendees will understand how racial biases cloud the investigation of missing persons cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that racial biases have been documented in the investigation of missing persons cases and that investigations of unidentified decedents do not always proceed equitably.

Racial bias permeates policing practices, investigative efforts, and the criminal justice system in variety of ways. For example, there is copious evidence that demonstrates that Black, Indigenous, and People of Color (BIPOC) are disproportionately treated during encounters with the police, whether those encounters be relatively benign (i.e., waiting on word that your missing child has been recovered) or confrontational (i.e., during arrest). Copious data demonstrate the results of such racial bias from BIPOC communities having a higher police presence, to disproportionate levels of arrest, violence, and death at the hands of police against Black people (especially men) vs. White people. Such bias even extends to the way BIPOC children are treated: arrested children are disproportionately Black and are sentenced as adults at a higher rate than White children defendants, and missing children are recovered faster when they are White. Women are not immune either; as one example, the epidemic of sex trafficking and assault against Native American women and girls goes relatively underinvestigated. Further, research has demonstrated that violence at the hands of law enforcement directed against Black people (to include children) is associated with a worldview of Black inferiority.

Biases have also been documented in investigative efforts related to missing persons cases where the social phenomenon of “missing White woman syndrome” shapes the exposure, engagement, and awareness of the public in relation to people who are missing. Although a growing body of literature establishes a notable racial disparity in the news coverage of missing individuals in the United States, much less is known regarding the impact this has on the investigation of missing persons cases, particularly in relation to the coroner/medical examiner system and human identification efforts.

This presentation aims to delve into this racial bias of missing and unidentified remains via an interdisciplinary perspective that combines expertise in criminology, forensic anthropology, and missing persons specifically. Through the combination of these disciplines, this study aims to further examine the ways in which race (particularly Black individuals compared to White individuals) shapes unidentified and missing persons investigations. The goals of this project include a better understanding of the role of racial bias in the ways in which missing persons cases are investigated, the resources allocated, and the likelihood of identification or recovery. This project is exploratory in nature; therefore, findings aim to serve as the building blocks in a better understanding of one of the many ways in which racial bias permeates the justice system. Previous research suggests that “missing White woman syndrome” is reflective in traditional news media and in the social media realm as well. Current research suggests that practices of law enforcement agencies reflect this racial bias as well. Suggestions for policies and future research are also explored, from both forensic anthropology and criminology perspectives.

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## Missing Persons, Racial Bias, Unidentified Remains



## Y7 The Limits of Blood Pattern Analysis (BPA) in the Analysis of a Forensic Case

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**Learning Overview:** After attending this presentation, attendees will be able to understand the founding principles of BPA and its practical application in a crime simulation case. Attendees will understand the ways BPA is applied and which responses it is able to give thanks to a careful, meticulous, and scrupulous inspection of the crime scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usefulness of the BPA principles known at the time of drafting this presentation, which aspects of it can be effectively adopted and which ones require implementation or integration with other techniques or methods in order to guarantee an effective, rapid, truthful, reliable, and expendable response in the investigative activity, not only of the forensic pathologist, but also of the detective, the criminologist, and any figure interested in the crime scene investigation.

The biophysical characteristics of blood make it unique among biological liquids. In particular, its composition and its cellularity ensure that it has a specific behavior in every dynamic capable of determining the more or less rapid encounter with a surface.

Since blood traces can be found with high frequency in the crime scene, the knowledge on how these traces can be distributed according to the ways in which the crime took place can be useful if not decisive in the reconstruction of the facts. Such knowledge and skills fall under the term BPA. The trajectory delineated by a high-speed blood jet on a smooth wall or the fall of drops to the ground are just two examples of how the blood can show itself to the forensic pathologist called for an inspection: although BPA can't determine whether it is actually a blood trace or not, nor establish precisely the origin, whether it was a spontaneous or simulated phenomenon, a key feature of BPA is that it can already determine in a scientifically validated manner many characteristics of the crime scene without using the aid of chemical-physical, genetic, or toxicological analyses of the blood fluid based on purely physical principles.

This study was able to appreciate the usefulness of the principles of BPA in an inspection that took place in the city of Lamezia Terme, Catanzaro, Italy. A man requested help from emergency services, reporting a suicide attempt due to a stab wound. Upon arrival of first aid at the house, the man, who was still alive, was seen as having a puncture wound on the back of his hand of little significance and entity, difficult to connect to the impairment of vital functions. It was also evident that the second floor of the house itself and the stairs to reach it were stained with a liquid that, at first sight, was of a blood nature to an extent obviously incompatible with the timing of bleeding and maintenance of the subject's vital functions as well as with the wound itself. In particular, the room in which he was waiting for help had the walls and the floor copiously smeared with reddish liquid. Faced with the obvious discrepancy between the amount of blood found in the rooms and the subject's wound, considering that the subject's former partner, who was pregnant, was unreachable, and being unable to establish the hematic nature (or not) of the congealed material on the walls and floor and its origin through chemical-physical and genetic study, the rescuers' suspicions were oriented on the murder of the ex-partner by the subject in question with simultaneous wounding of the back of his hand in a hypothetical struggle, and the consequent concealment of the corpse.

The inspection of the crime scene, aimed at inspecting and collecting multiple biological traces, allowed the team, with careful observation based on the principles of BPA, to suspect the artificial nature of true blood traces, the staging of the entire scene, and the suicidal nature of the facts. The genetic and toxicological studies performed by the Carabinieri Unit of Special Investigations based in Messina, Italy, fundamental and necessary for confirmation, confirmed the team's suspicions.

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### Forensic Sciences, BPA, Simulation



# Young Forensic Scientists Forum Posters – 2021

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## Y8 A Landscape Study of Rapid DNA Technology

*Hannah M. Cawley\*, Pittsburgh, PA 15210; Lyndsie N. Ferrara, PhD, Duquesne University, Pittsburgh, PA 15219*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of Rapid DNA technology and its application.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing together published information as well as user interviews to provide the community with a resource for better understanding Rapid DNA technology.

Rapid DNA machines allow a single-source DNA sample to be processed in approximately 90 minutes, compared to the traditional analysis method that can take anywhere from days to weeks in a forensic laboratory. Being able to produce a DNA profile in less than two hours is significant because it can help to address the issue of backlog in the criminal justice system, provide leads to investigators, and bring closure to loved ones of mass disaster victims. All trained personnel have to do is collect the sample and insert it into the machine cartridge. The extraction, amplification, separation, detection, and allele calling are all done by the machine. If a usable profile is produced, it is compared to local and sometimes national databases in order to obtain a “hit” or match. Because forensic scientists are not needed for the analysis, Rapid DNA started as a quick screening tool to be used in police stations to compare arrestee reference samples (saliva swabs) to those DNA databases. As the technology continued to be implemented in various agencies, it has evolved to process additional media, including blood, bone, semen, and tissue samples.

The goal of this study was to compile the extensive information about Rapid DNA technology that is spread out across various sources. It also incorporated user experiences in order to show different perspectives. This is meant to be an easily accessible resource for agencies interested in adopting Rapid DNA technology and for professors and scientists that are interested in learning more or teaching about it. All information was sourced from interviews, webinars, websites, and scientific journals. There were three interviews conducted to highlight some different perspectives in the field. The first was with Anna Dadhania, a forensic scientist and administrator for the Orange County District Attorney (OCDA) DNA database, who works for the OCDA’s office in California. The next interview was with Dr. Richard Selden, the founder and Chief Scientific Officer of ANDE Corporation, which is the leading developer for Rapid DNA technology and devices. The final interview was with Fred Harran, the Director of Public Safety for the Bensalem, PA, police department, who played a key role in implementing the nation’s first countywide DNA database. Along with these testimonies, the research compiled information about the available Rapid DNA platforms, protocols set by the Federal Bureau of Investigation (FBI) regarding the use, who is using the technology, and how it is being used across different agencies. Public concerns about the reliability of the technology and laws surrounding DNA collection are addressed as well.

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### DNA Analysis, Rapid DNA, Forensic Investigation



## Y9 A Comparison of Semi-Automated and Manual Differential Separation Methods for Mock Sexual Assault Swabs

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**Learning Overview:** After attending this presentation, attendees will be familiar with the SpermX™ differential separation method and understand how this novel, manual procedure compares to the semi-automated differential separation protocol of the Federal Bureau of Investigation (FBI) Laboratory Unit when used to extract male and female DNA from mock sexual assault samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how new developments in DNA technology can be evaluated by comparing alternative procedures with validated protocols.

In the event of a sexual assault, separation of suspect from victim DNA is possible through differential extraction if the evidentiary mixture consists of female epithelial cells and male spermatozoa. The basis of differential extraction is the differing properties of epithelial and sperm cell membranes, specifically the comparative sturdiness of the proteins making up the sperm head containing the male DNA. Effective retrieval and separation of male and female DNA fractions is important in generating high-quality Short Tandem Repeat (STR) profiles that can be utilized in identifying suspect(s) in a sexual assault case.

Differential extraction has been conventionally performed using Proteinase K (PK) to lyse epithelial cells, then Dithiothreitol (DTT) to lyse sperm cells after the fractions were separated through centrifugation. The protocol for differential separation currently used by the FBI Laboratory is a semi-automated variation of this method, with the use of the QIAcube® for separation of female and male cells, and the EZ1™ Advanced XL for DNA purification. SpermX™ is a manual differential separation method that uses a novel nanofiber matrix to separate sperm from epithelial cells. As a cellular mixture is washed through the SpermX™ device, epithelial DNA lysate will flow through while sperm cells are trapped within the matrix. Sperm DNA is released using a sperm digest buffer and extracted with the EZ1™ Advanced XL. Comparison of the FBI and SpermX™ methods through mock sexual assault sample extraction may indicate an alternative differential method that can be used if the current automation is unavailable.

A solution of female epithelial cells was prepared by washing neat saliva and resuspending the resulting pellet in Tris-EDTA (TE) buffer. Human semen from five different male donors, acquired from a commercial source, was diluted 1:20, and equal volumes of epithelial solution and semen dilution were evenly distributed onto cotton swabs. Mock sexual assault samples were quantified with Quantifiler® Trio. Following quantification, samples were amplified with GlobalFiler® Polymerase Chain Reaction (PCR) Amplification Kit and genotyped using the 3500xL Genetic Analyzer. Short Tandem Repeat (STR) profiles were analyzed using GeneMapper® IDX v1.6.

Comparisons between the FBI and SpermX™ differential protocols were made from the samples' DNA quantity, profile quality, and degradation level for each of the male and female differential fractions. The female fractions of both methods were similar in containing a mixture of male and female DNA. They also both yielded high-quality, non-degraded DNA as indicated by their degradation indices, which were 1.04 and 1.00 for the FBI method and SpermX™, respectively. There was no significant difference between the female fraction DNA quantities, which ranged from 0.14 to 5.32ng/uL for the FBI method and 1.57 to 6.20ng/uL for SpermX™ ( $p > 0.05$ ). Therefore, the FBI and SpermX™ differential methods are comparable when extracting female DNA from a mock sexual assault swab.

When observing the male fraction, the FBI and SpermX™ methods were similar in separation success and DNA quality. Both methods yielded clean male fractions, showing success in differential separation. The degradation indices of the male fractions were 0.79 for the FBI method and 0.78 for SpermTrap™, which indicates high-quality, non-degraded DNA. Unlike the female fractions, there was a significant difference in male DNA quantity. The DNA quantity extracted by the FBI method ranged from 0.14 to 5.32ng/uL, which was significantly larger than the quantity of male DNA extracted by the SpermX™ method, which ranged from 0.04 to 2.02ng/uL ( $p < 0.05$ ). Although both differential extraction methods produced a well-separated, high-quality male fraction, the FBI method was able to extract a higher quantity of male DNA from mock sexual assault swabs.

The DNA analysis of mock sexual assault swabs enabled the comparison of two differential separation methods, showing the FBI and SpermX™ methods are of comparable quantity and quality.

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### Differential Extraction, SpermX™, STR Profile



# Young Forensic Scientists Forum Posters – 2021

## Y10 Firearm and Toolmark Standard Operating Procedures (SOP) Assessed to Determine Variety in Forensics Laboratories

Madison McGregor\*, Columbia, MO 65202-2470

**Learning Overview:** The goal of the presentation is to show there is still a need for standardization of laboratory manuals in the firearm and toolmark discipline.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that much variability is still prevalent between laboratories, which can in turn affect how cases are tried on a state-to-state basis.

This study collected Standard Operating Procedures (SOPs) from a small sample of labs from across the United States. This study focused on requirements of firearm and toolmark examiners and how they differ from proposed Organization of Scientific Area Committees (OSAC) standards that are currently under discussion. The 2009 National Academy of Sciences (NAS) Report *Strengthening Forensic Science in the United States: A Path Forward* called for improvement in quality assurance standards in forensic science over a decade ago, showing a need for effective SOPs in laboratories across the United States.<sup>1</sup> This, combined with the fact that firearm and toolmark examination has been accepted in the courts since the 1920s, suggests that practices by this time should be similar across labs.<sup>2,3</sup> A total of 15 laboratories (L1–L15) were selected for the study and five areas were determined for focus in the SOPs. These areas included examination of bullets, firearms, cartridges, toolmarks, and tools. The reference used for comparison was proposed standards by OSAC for firearm examination. The research also aims to include the opinions of examiners to ensure that all standards used are ones that examiners agree to be relevant.

Bullet examination appeared to have the best overall agreement; because of the five core areas selected, it contained the most SOP information at 92% of OSAC proposed guidelines being met. L4 had an SOP with all guidelines in the proposed OSAC standards met. L9 diverged in 19 proposed OSAC guidelines, while most labs averaged nine differences overall. The 15 labs in total had 136 differences from the core requirements.

Data from the FORESIGHT project showed a wide range of financial allocation for labs from \$187 to \$1,604,830.<sup>4</sup> This along with the fact that cases for some labs in the firearm discipline were as low as 12 a year, with less than one full-time employee dedicated to firearm and toolmark work alone could be why some laboratory manuals lacked detail.<sup>4</sup> Standardization of lab manuals—at least for procedures that are deemed to be critically important—can help ensure that firearm and toolmark evidence is interpreted uniformly regardless of location and can provide strong support for examiners while testifying in court.<sup>5</sup> The standards under discussion in the OSAC are likely to be revised and refined in months to come. Once a set of standard SOP recommendations is adopted, however, it will be possible to ensure common practice across the nation.

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### Firearm, Operating Procedures, Toolmark



# Young Forensic Scientists Forum Posters – 2021

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**Y11 The Quantification of Persistent Organic Pollutants in Organic and Non-Organic Produce Using Stir-Bar Sorptive Extraction With Gas Chromatography/Mass Spectrometry (GC/MS) and Isotope Dilution Mass Spectrometry**

*Tiffany L. Hoke\*, Carnegie, PA 15106*

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**WITHDRAWN**



# Young Forensic Scientists Forum Posters – 2021

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## **Y12 A Case Study of the Correlation Between Bullying and Subsequent School Shootings**

*Levi E. Peck\**, Cameron University Undergraduate Research Team, Duncan, OK 73533; *Hunter N. Gault\**, Cameron University Undergraduate Research Team, Elgin, OK 73538; *Rikki A. Tasso-Thompson\**, Cameron University Undergraduate Research Team, Cache, OK 73527; *Christina A. Leija, MS, San Antonio, TX 78227*

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**WITHDRAWN**



## Y13 An Unintentional Drowning With a Contributing Factor of Carbon Monoxide Intoxication

Raymond H. Bayer III, BS\*, Western Michigan Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; Joseph A. Prahlow, MD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

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**Learning Overview:** The goal of this presentation is to present a case of carbon monoxide exposure contributing to a drowning death of a recreational boater.

**Impact on the Forensic Science Community:** This case will impact the forensic science community by serving as a public health warning on the dangers of carbon monoxide emission from boats and its potential role in contributing to drowning deaths.

There are approximately 4,200 cases of drowning in the United States every year.<sup>1</sup> Commonly identified risk factors for drowning include male sex, alcohol use, and lack of a life jacket.<sup>2</sup> This case report will examine a less commonly identified risk factor that should be considered for drowning deaths: carbon monoxide exposure.

A 19-year-old female drowned after entering a lake from a boat in which she had been riding. Prior to entering the water, she had reported a cramp and had been riding in the boat at low speed for two hours. She had been sitting on the right back side of the boat directly behind the driver's seat. A passenger reported that she had had two beers that evening. According to other passengers on the boat, she entered the water to urinate and was unable to be located shortly afterward. One passenger reported seeing her head tilt back, then she went underwater without coming back up. The passengers called 911 and unsuccessfully searched for her in the water. The local dive team responded and located her body in the water, approximately three hours after she went missing.

Autopsy revealed muddy water in the mouth, nose, and upper airways, muddy fluid in the trachea and bronchi, and focal hemorrhages in the lungs bilaterally. In addition, bright red lividity and musculature were noted, and the leptomeninges were a bright red-pink. Toxicology testing of postmortem blood revealed an alcohol level of 71mg/dl and a carboxyhemoglobin level of 46.2%. The cause of death was determined to be drowning with a contributory factor of carbon monoxide intoxication.

Carbon monoxide levels on idling and stationary boats frequently exceed 1,000ppm in the rear deck area, enough to cause loss of consciousness within two hours of exposure.<sup>3,4</sup> The addition of a catalytic converter to boat engines can reduce carbon monoxide concentrations by up to 90%. The ability of carbon monoxide intoxication to produce loss of consciousness makes it especially dangerous to persons on or near recreational boats. Recreational boaters in general, and owners of older model boats without catalytic converters in particular, should be made aware of the danger of their engine as a source of carbon monoxide. They should be encouraged to install carbon monoxide detectors. In addition, forensic pathologists should consider carbon monoxide exposure as a possible contributing factor in drowning deaths in situations where such exposure may have occurred.

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### Carbon Monoxide, Drowning, Drowning Risk Factors



## Y14 Determining the Ideal Swab Type for Collection of the Microbiome for Forensic Identification Purposes

Natalie M. Wise, BS\*, Westlake, OH 44145

**Learning Overview:** The goal of this presentation is to educate attendees on the human microbiome, a newly emerging forensic tool, and the swab type that is most ideal for its efficient collection and elution for further analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a recommendation regarding the best swab type for collection of the human microbiome, as well as the potential for recovery of the microbiome off of various surfaces. The information could be valuable for future researchers who continue to investigate the potential forensic applications of the human microbiome, including studies investigating recovery from additional surfaces and from human subjects.

In recent years, forensic scientists have begun looking at the human microbiome as a new tool in forensic science. The microbiome is made up of all the microorganisms living on or in the human body.<sup>1</sup> Past studies have shown that it may be possible to use the microbiome as a unique identifier, to link cohabiting individuals, or even to connect a person with a location.<sup>2-5</sup> The relative abundance of the microbiome shows potential for it to be used in cases where a usable human DNA sample is not left at the scene.<sup>6</sup> In order to study the microbiome, one must first be able to successfully collect it, then release it from that collection tool for further examination. This may sound simple, but this area of research is so new that even the best method of collection has not yet been determined.

Therefore, this research focuses on finding the optimal swab type for collection and analysis of the human microbiome. To do this, a predetermined amount of a bacterium, *Proteus mirabilis*, will be deposited directly onto four swab types (traditional cotton, nylon flocked, superfine dental applicators, and Luna dissolvable swabs). Then, extraction of the bacterial DNA will be performed for all swabs using the MagMAX™ Total Nucleic Acid Isolation Kit, followed by quantitation using real-time Polymerase Chain Reaction (PCR). This will allow for determination of absolute microbial DNA recovery and comparison of yields across the four swab types. An additional set of samples will be taken through the same extraction and quantification as the swab samples but with no swab used. These positive controls with manipulation will allow for determination of how much microbial DNA is lost during the extraction process rather than being left behind in the swab. Following the initial evaluation of the four swab types directly impregnated with the bacteria, the two top performing swabs will be evaluated further by adding the additional step of picking up the bacteria from various surfaces representative of common household materials (wood, glass, metal) before extraction and quantification.

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### Microbiome, Quantitative PCR, Swab



## Y15 Qualtrics Survey of Gamma-Hydroxybutyric Acid (GHB) Methodologies in Blood and Urine

Jeovanna S. Badson, BS\*, Brooklyn, NY 11203; Marc A. LeBeau, PhD, Federal Bureau of Investigation Laboratory, Quantico, VA 22135; Camila Berner, Kura Biotech, Perto Varas N/A, CHILE; Jack Andrews, Kura Biotech, Rancho Dominguez, CA 90220; Karen S. Scott, PhD, Arcadia University, Glenside, PA 19038

**Learning Overview:** After attending this presentation, attendees will have an increased understanding of the lack of general consensus that laboratories across the United States have regarding the analysis and cut-offs of GHB.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing survey data on toxicology laboratory analysis of GHB and its reported cut-offs.

GHB is an endogenous short-chain fatty acid that depresses the Central Nervous System (CNS) via GHB receptors in the brain. GHB also works via GABA<sub>B</sub> receptors. GHB's popularity increased due to bodybuilders who used it to improve athletic performance.<sup>1</sup> Due to its anesthetic property, it became suspected of use in Drug-Facilitated Crimes (DFC).<sup>1-4</sup> As such, in March 2000, GHB became a federally controlled Schedule I drug in the United States.<sup>1</sup> Exogenous GHB has a narrow detection window of 3–6 hours in blood and up to 12 hours in urine.<sup>5</sup> It is important that biological samples be collected very quickly to enable differentiation of exogenous from endogenous GHB levels.

This presentation illustrates the collation and comparison of methods used for the analysis of GHB in blood and urine, as well as the cut-offs used in laboratories across the United States. The scientific literature contains a wide range of methods and cut-offs, so the goal was to collect data obtained from a survey to determine the most commonly used methodologies for GHB analysis and the cut-offs for reporting in order to establish a best practice for GHB determinations.

The survey was sent to toxicology laboratory directors worldwide and was created using the web-based survey tool, Qualtrics, which allows users to collect, analyze, and present survey data.

The majority of respondents were from government laboratories (57%) within the United States with an even split of casework between Driving Under the Influence of Drugs (DUID), Postmortem (PM), and DFC. Approximately 60% of the responding laboratories performed testing for GHB. More than 90% of the laboratories testing for GHB ( $n=46$ ) reported conducting analyses in-house, with the majority of the testing being performed for DFC cases. Only four laboratories (10%) hydrolyze their urine samples prior to analysis. The majority did not force a GHB: Gamma-Butyrolactone (GBL) equilibrium through pH manipulation during analysis. There was a 60:40 split of laboratories who derivatized GHB samples and about 60% of laboratories reported conducting analysis by Gas Chromatography/Mass Spectrometry (GC/MS) or Gas Chromatography/Flame Ionization Detector (GC/FID). None of the laboratories analyzed for GHB in its conjugated form. Cut-off levels ranged from 1mg/L–50mg/L in blood and urine with the majority of respondents using either 5mg/L or 10mg/L. Either aqueous- or matrix-spiked standard were reported for calibration in both matrices.

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### GHB, Survey, Blood and Urine



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Chiara Della Rocca - B63  
Kaeleigh DeMeter, BS - H26  
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Sara Dempsey, PhD - K33  
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Emily A. DeRuyter - B97  
Vincent J. Desiderio, Jr., MS - S02  
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E52, E57, F10, H59, H71  
João De Sousa, MS - E43  
Sylvain Desranleau, DMD - G10  
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Cemyigit Deveci, MD - H108  
Gregory M. Dickinson, MD - H40  
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A110, Y6  
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Meaghan C. Dougher, MPS - H138  
Nancy R. Downing, PhD - E23  
J.C.U. Downs, MD - H41, W14  
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Rhian Dunn, MS - A41  
Tomasz Dziedzic, PhD - J16

## E

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Massimiliano Esposito, MD - H43, H87,  
H104  
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Uwom O. Eze, MD - E84

## F

Mylène Falardeau, BSc - B111, J35  
Amin Fallahi, BS - C26  
Samuel J. Farris, BS - H64  
Rolf Fauser - J30  
Laney R. Feeser, MA - A87  
Alan R. Felthous, MD - I17, I18  
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Khalid S. Feras, MPhil - B99  
Nicole Fernandez Tejero, BS - B71  
Michela Ferrara, MD - E49, E50, E51,  
E52, E57, F10, H59, H71  
Morgan J. Ferrell, MA - E2  
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Angela G. Fielder, MA - I19  
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Tais R. Fiorentin, PhD - B22  
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W05  
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Jonathan M. Ford, PhD - H139  
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Amber Fortney - E74  
Kristen L. Fowble, PhD - B145  
Bryanna Fox, PhD - Y6  
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Virginia Franqueira, PhD - W03  
Eric Frauenhofer - A18  
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# Presenting Author Financial Disclosure—2021

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Laura C. Fulginiti, PhD - A2  
Kaitlyn J. Fulp, BA - A74

## G

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Shawna F. Gallegos - B115  
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Heather M. Garvin, PhD - A52  
Cynthia R. Garza, JD - F1  
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Irina Geiman, MS - CB5  
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Zeno J. Geradts, PhD - W13, W14  
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Simone Gittelson, PhD - W10  
Lorenzo Gitto, MD - H9, H55, H79  
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Erin M. Gorden, MFS - W15  
Jan M. Gorniak, DO - W14  
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Zygmunt M. Gorski, BA - D18  
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Mete K. Gulmen, PhD, MD - E26, H62  
Pramod Gumpeni, MD - H26  
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Carlos A. Gutierrez, MSFS - E27  
Barbara Guttman, BA - C8, W03  
Keroshini Guynes - A5

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Robert F. Hedges, JD - F24  
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## I

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# Presenting Author Financial Disclosure—2021

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Yungchun Lai - B44  
Victoria Lamond, BA - A47, A59  
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Tiffany R. Layne, MS - B1  
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Federico Manetti, MD - H91, K21  
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Sophia Mavroudas, MA - A31  
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Kenneth E. Melson, JD - W09  
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Vadim Mesli, MD - A72  
Diana Lynn Messer, PhD - A90  
Hacer Merve Meydanci - E53  
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Emily F. Michon, MA - A39  
Robert A. Middleberg, PhD - K35, K53  
Kari M. Midthun, PhD - K52  
James Millette, PhD - D17  
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Mohammad M. Mirza, MS - C27  
Randolph L. Mitchell, DMD - G7  
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Arun S. Moorthy, PhD - B17, B18, W07  
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Sharon K. Moses, PhD - E25  
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Patricia Mullaney, BS - W03  
Jerome Mulloor, MS - B83



# Presenting Author Financial Disclosure—2021

## N

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## O

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Nicole R. Odom, MSFS - W03  
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## P

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Andrea Palmiotto, PhD - A51  
Kaitlyn B. Palmquist, BS - K39  
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Nicolette Parr, PhD - A104  
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Nicholas V. Passalacqua, PhD - A40  
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Mark W. Perlin, PhD, MD - S01  
Anja Victoria Perner, BSc - H120  
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Michael Pertsinakis - J15  
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Amy Pham, BA - A88  
Angelina I. Phillips, MD - H75, H76, H111  
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Caitlin E. Porterfield, MS - E74  
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Noemi Procopio, PhD - B131  
Meghan Prusinowski, MS - B95

## Q

Alessia Quattrocchi - H122  
Aline Quenault - I25

## R

Davide Radaelli - H70  
Roberto Raffaele, BE - E63, H47, Y7  
Joseph M. Rahm, BS - B74  
Christopher Ramos, MD - E85  
Katherine Ramsland, PhD - E70  
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Cristina L. Rentas, MFS - B133

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Dayla C. Rich, BS - E4  
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Andreas J. Rippert - J30  
Henry L. Risemberg, MS - C29  
Katherine A. Roberts, PhD - F11  
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Marcus Rogers, PhD - C7  
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Jarrett P. Ross, BA - B36  
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Walter F. Rowe, PhD - B156, J32  
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Leonid I. Rudin, PhD - W13  
Justin Rueckert, DO - H107  
Sittiporn Ruengdit, PhD - A29  
Teresa M. Runge, MS - A30  
Michael Russ, MS - E22  
David Russell, MS - B72  
Lenora N. Rutten - J33

## S

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Matteo A. Sacco, MD - E61, E62, E66, E67, H23, H46, H60, H68, H74, H88, H93, I20, Y7  
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# Presenting Author Financial Disclosure—2021

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Madeleine J. Swortwood, PhD - W11

## T

Milazim Tahirukaj - B98  
Tobin A. Tanaka, BS - J34, J35, S02  
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## U

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## V

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Muskan Vir - J35  
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---

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---

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---

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Carlos J. Zambrano, PhD - A65  
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Xin Zhang, MD - H99  
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E69, H60, H93, H105, Y7  
Sotirios Ziogos, BSc - H113  
Joel A. Zlotnick, MSFS - W16  
Tong Zou, MS - D2

#

#DataStories-F23

Δ

Δ<sup>9</sup>-THC-D28

B

BAPP-H42

3

3D Analysis-A11

3D Digital Microscopes-J5

3D Imaging-A33, A34

3D Modeling-A48, A49, F12

3D Prints-A107

3D Scans-A47

A

Abnormalities-J17

Absolute Quantification-B60

Absorbent Materials-B142

Abusive Head Trauma-H5, H103

Accessibility-D15

Accident-E81

Accuracy-A12, A75, A115, E14

Activity-F14

Activity Level-B111

Activity Level Proposition-F8

Actual Innocence-F1

Acute Intoxication-H112

Addiction-E53

Additive Manufacturing-J35

ADF Oxycodone-K52

Adhesive-E9

Adhesive Analysis-B101

Adipositas Cordis-H105

Admissibility-F21

Admixture-A113

Adolescent-H6

African American-A18

Age-at-Death-A27, A31

Age-at-Death Estimation-A32, Y1

Age Determination-A105, B67

Age Estimation-A20, A21, A22, A23, A25, A26, A28, A29, A78, G17, G18, G22

Age of Bloodstain-B4

Age Study-A30

Agency Capture-D19, D21

AI (Artificial Intelligence)-C5

Air Embolism-H4

AJPA-A41

Allele Frequencies-B136

Alternate Light Source-B61, E23

Alternate Workflow-B130

Alternative Light Source-E36

Alternative Specimen-K51

Alveolar Ridge-A11

Ambush-E58

Ammunition-H53

Amok Syndrome-I23

Amplification-B76

Analytical Chemistry-B115

Anaphylactic Death-H43

Ancestry-A35, A102, A103, A109, A110, A113, A114, A115

Ancestry Estimation-A36, A111

Ancestry Informative Markers (AIMs)-B121

Android™-C3, C10

Android™ Apps-C17

Android™ Malware-C13

Anesthetic Drug-H92

Anesthetic Inhalation-H71

Angiectasias-H63

Animal Cruelty-I6

Anomalous Origin of Right Coronar-H93

Anthropology-A12, A58, A75, E21

Anthropology Population Data-A20

Anti-Impulsive Aggressive Agent-I28

Antipsychotic Drugs-H87

Aorto-Esophageal Fistula-H100

Applicability-A25

Aquatic-D4

Arad Corpus-J8

Arc-H38

Armed Conflict-A108

Arrhythmia-H112

Arson Collection Materials-B142

Artifact Analysis-C9

Artificial Fingerprints-B157

Artificial Intelligence-G10

Asbestos-D17

Asia and Pacific-A85

Asphyxia-H69

Asphyxiation-H144

Assassination-B107

Assessment-A42, W18

Asylum-I1

Atomic Spectroscopy-B101

Attachment Type-I7

Attempted Suicide-Y5

Attribution-D9

Audio-C10

Audio Authentication-C11

Audio Enhancement-C5

Audio Recording Analysis-H133

Augmented Reality-E70

Authentic Hair Reference Material-K26

Autism Spectrum Disorder (ASD)-H87

Autoerotic Death-E82

Automatic Text Classification-D30

Automotive Safety Defects-D21, D24

Autopsy-E51, H2, H10, H23, H31, H55, H75, H80, H85, H95, H111, H122

Autopsy Findings-H26

Autopsy Investigation-H49, H91, H92, H108

Avoidance-E7

B

Bacteria-B70

Bad Science-F18

Bail-CB3

Bail Elimination-CB3

Bail Reform-CB3

Ballistic Gelatin-H135

Ballistics-A1

Ballistics Expert-B100

Basilicata Data-E54

Battery Ingestion-H100

Bayes Factor-A81

Bayes' Theorem-F26

Bayesian Framework-W02

Benign Cardiac Tumor-H82

Benzodiazepine-E89, K48

Benzodiazepines-B16

Best Practice Manual-J16

Beverages-B79

Bias-B138, I4

Biasability-A43

Biblical Inscriptions-J8

Biocultural Profile-A39

Bioerosion-A71

Biogeographical Ancestry-B63

Bioimetric-J12

Bioinformatics-B46

Biologic Profiling-E13

Biological Evidence-E26

Biological Profile-A15, A75, A76, A99, A111

- Biological Variation-A17  
 Biomechanics-D16, E20  
 Biometric-I16, J14, J15, W01  
 Biometric Security-C26  
 Birt-Hogg-Dubé Syndrome-H80  
 Bite Injuries-H137  
 Bitemark-G15  
 Bitemarks-CB4  
 Black Box-B108  
 Black Box Study-J1  
 Black Esophagus-H109  
 Bladder Wash-K51  
 Blind Proficiency Testing-B24  
 Blind Quality Control-E80  
 Blind Testing-E80  
 Blind Transcript Review-B118  
 Blood-B32, K3, K49  
 Blood and Urine-Y15  
 Blood Concentration-K28  
 Blood Pool-B119  
 Bloodstain-B4, B5  
 Bloodstain Pattern Analysis-D2  
 Blow Fly-A95, H19, H22  
 Blunt Force Trauma-A91, H140  
 Blunt Impact Load-A89  
 BMI (Body Mass Index)-H89  
 Body Fluid ID-B6  
 Body Fluid Identification-B1  
 Body Position-A63  
 Body-Worn Cameras-C21  
 Bone-A60, A94  
 Bone Bioerosion-H14  
 Bone Diagenesis-A71  
 Bone Shrinkage-A61  
 Borderline Personality Disorder-I9  
 Bounce House-H98  
 BPA-Y7  
 Brain Injury-D16  
 Brain Patterning-E25  
 Breath Analysis-D27  
 Bromazepam-E89  
 Brophine-H9  
 Bruise-E23  
 Bullet-B107  
 Bullet Hole Depth-B110  
 Bullet Topography Measurements-B109  
 Bullying-Y12  
 Bupropion-K10  
 Burial-E21  
 Burn-H38  
 Burned Human Bone-A98  
 Burned Human Remains-A97  
 Burned Victims-G8  
 Burnt-G12  
 Business Development-J22  
 BWC Video Analysis-C22
- ## C
- 
- Cable Ties-H44  
 Cadaver-H128  
 Cadre-E6  
 Caffeine-H112  
 Calcium/Phosphorus Ratio-Y1  
*Calliphora Vicina*-H21  
 Calliphoridae-H18  
 Canine-K8  
 Canine Detection-B96, E1  
 Canines-F27  
 Cannabinoids-B14, B79, K16  
 Cannabis-B13, B83, D3, D28, K44, K46, W17  
*Cannabis Sativa*-B134  
 Capillary Electrophoresis-B121, B126  
 Capsaicin-B104  
 Carbon Monoxide-Y13  
 Carbon Monoxide Poisoning-D12  
 Carbonization-E52  
 Carboxyhemoglobin-K18  
 Cardiac Hamartoma-H82  
 Cardiopulmonary Resuscitation-A3, H32  
 Cardiovascular-H33  
 Carpal Bones-A22  
 Cartridge Cases-B62  
 Case Coordination-B8  
 Case Law-D25, D26  
 Case Processing-B117  
 Case Study-B56  
 Caseload-A40  
 Casework-J13  
 Caspase 3-H130  
 Caspase 9-H130  
 Cassowary-H65  
 Cathinones-K36  
 Cattle-H66  
 Cause of Death-H24, H30  
 CBD-K45  
 CCTV-E55  
 Cell Differentiation-B10  
 Cellphone-C9  
 Cementum Annuli-G4  
 Cemetery-A68  
 CFReDS-C23  
 Chart-J10  
 Cheiloscopy-F25  
 Chemical Waste Treatment-B144  
 Chemometrics-B15, B27, B113, B143  
 Child-H6  
 Child Abuse-A90, E67, H2, H3, H99, H145, I26  
 Child Neglect-I11  
 Child Rights-E32  
 Child Sexual Abuse-E32  
 Childhood-H62  
 Children-G18  
 China-LW1  
 Chiral Analysis-K42  
 Chloroquine-B84  
 Choking-H69  
 Chromatography-B83  
 Chronic Alcohol Abuse-H106  
 Chronic Diseases-I11  
 Chronic Ischemic Brain Injury-H5  
 Chronic Lymphocytic Leukemia-H79  
 Clandestine Laboratory-B31  
 Classification-D2  
 Classification System-A97  
 Clinical Forensic Medicine-H145  
 Close Non-Match-B116  
 Close-Range Photogrammetry-E2  
 Clothing Color-H22  
 Cloud-C24  
 Cloud Data-C15  
 Cocaine-H109  
 Code Writing-J21  
 Codes-D25, D26  
 CODIS-CB2, H131  
 Cognitive Bias-A44, A45  
 Cold Case-B36, E62, E74, H126  
 Cold Cases-E29  
 Cold Temperatures-A73  
 CollectEject™-B65  
 Collection-B74  
 Collision Energy-B98  
 Colorimetric-B1  
 Colorimetric Sensors-E34  
 Combining Evidence-B38  
 Commingling-A54  
 Communication Channels-J30  
 Communism Victims-A9  
 Comparison Language-B29  
 Compelled Decryption-F20  
 Competency-W18  
 Complex Suicide-E59, Y4  
 Compression-A88  
 Computational Analysis-J7



- Computed Tomography-A10, A21, A23, A26, H140  
Cone Beam Computed Tomography-G6  
Confinement-I21  
Conflict of Interest-H124  
Congenital Diaphragmatic Hernia-H98  
Consumption-B66  
Contamination-B112  
Continuing Education-E77  
Contraband Firearms-B96  
Conventional Radiography-A78  
Conviction Integrity-F1  
Coronavirus (SARS-CoV-2, COVID-19)-H27  
Coroner or Sheriff-Coroner Systems-H124  
Corpse Concealment-E52  
Corpse Relocation-A95  
Corrupted Evidence-F18  
Cortical Porosity-A34  
Counterfactual Causation-H133  
Counterfeit-W16  
Courts-F28  
CoverMe-C12  
COVID-19-E84, F28, H23, H24, H25, H26, H28, H29, H85, I12, I20, K1, K23, W04  
CPR-A87, H32  
Cranial Fracture-A91  
Cranial Suture-A29  
Crash Locking Tongue-D20  
Credibility Assessment-E31  
Cremation-A64  
Crime Laboratory-B117  
Crime Prevention-E53  
Crime Scene-B8, B50, B119, E22, E38, E59  
Crime Scene Documentation-A59  
Crime Scene Examination-E58  
Crime Scene Investigation-B61, E10, E50  
Crime Scene Reconstruction-C32, F12  
Criminal Defense-F13  
Criminal Homicide-I6  
Crimtort-D19  
Critical Race Theory-A104  
Cross Reactivity-K10  
Crossbow Injury-E47  
Crowdsourcing Forensics-C28  
CSI Effect-F6  
Culture-I19  
Curriculum-E75  
Cutoffs-K27  
Cutting Agent-B20  
Cutting Agents-B22  
Cyanide-H108  
Cyanoacrylate Fuming-E35  
Cyber Forensics-C27
- D**
- DART®-HRMS-B27  
DART®-MS-B18, B88, W07  
Data Analysis-B162  
Data Augmentation-J23  
Database-B33, B58, F16, F17  
Dating Apps-E32  
DCS-J12, J14, W01  
Dead Body Search and Recovery-A85  
Death-E89, H9, H27, I15  
Death Certification-H141  
Death in Custody-H133  
Death Investigation-E30, H52, H124, H127  
Decapitation-H68  
Decedent Identity-A112  
Deception-D33  
Deception Detection-D30, D31  
Decision Reliability-E31  
Decision Theory-B140  
Decomposition-A63, A69, A73, A74, B154, E88, H16, H113, H117, LW4  
Decomposition Ecology-H15  
Decontamination-H129  
Decryption-C12  
Deep Learning-B103  
Deepfake-C2, W13  
Deepvoice-W13  
Defective Fresh Air Intake-D12  
Defense-I19  
Defense Sprays-B104  
Degradation-B76, B128  
Deliriant Induction-I22  
Delusion-I10  
Demirjian's Method-G20, G22  
Demonstrative Exhibit-J10  
Dental Age Assessment-G16  
Dental Age Estimation-G4, G16, G19  
Dental Anthropology-A36  
Dental Identification-G8  
Dental Sleep Medicine-G5  
Dental Tape-D17  
Deployable Instrumentation-B31  
DFSA-K33  
Diabetic Ketoacidosis-H110  
Diagenesis-A94  
Diagnostic Workflow-H43  
Diatom Test-H67  
DIC-H103  
Differential-C24  
Differential Decomposition-H114  
Differential Extraction-Y9  
DiGeorge Syndrome-H100  
Digital-C7, C29, J9, J11  
Digital Artifacts-C28  
Digital Audio Recorders-C11  
Digital Crime Scene Investigation-E70  
Digital Data Sets-C23  
Digital Data Sharing-A107  
Digital Evidence-C13, C19, F20, F23  
Digital Forensics-C23, W03  
Digital Image Forensics-C17  
Digital Modality-A24  
Digital Sorting-B45  
Digitally Captured Signatures-J16  
Direct Amplification-B55  
Direct MS-B82  
Direct-to-PCR Extraction-B49  
Disability-H62  
Disability Bias-F9  
Disappeared Persons-A108  
Disaster-H35  
Disaster Mortality-H36  
Disaster Statistics-H36  
Disaster Victim Identification-H139  
Disease Transmission-E7  
DNA-B2, B41, B47, B48, B56, B58, B122, B128, B150, E37, F14, F15, F16, F17, F22, H116, W10, W15  
DNA Analysis-B7, B44, B120, B126, H121, Y8  
DNA Barcoding-B134, H12  
DNA Collection-B53, B65  
DNA Degradation-B72  
DNA Discovery Standards-F13  
DNA Donor Age-F24  
DNA Extraction-B127, Y3  
DNA Identification-A86  
DNA Methylation-B71, F24  
DNA Mixtures-B38, B41, B44, B136, B138  
DNA Shedding Propensity-B9  
DNA Testing-F11  
DNA Transfer-B62  
Document Analysis-J20, J24  
Document Experts-J30  
Documentation-E22  
Documented Skeletal Series-A83



Documents-J17  
 Dog Bites-H137  
 Drawing-E25  
 Driving-K29  
 Driving While Intoxicated-K32  
 Drones-C16  
 Drowning-E60, F10, H67, Y4, Y13  
 Drowning Deaths-A85  
 Drowning Risk Factors-Y13  
 Drug-B31  
 Drug Abuse-Related Death-E51  
 Drug Analysis-B15, K11  
 Drug and Substance Use-K35  
 Drug Chemistry-B81  
 Drug-Facilitated Crime-K22  
 Drug-Facilitated Sexual Assault-K22  
 Drug Stability-K25  
 Drug Testing-K5  
 Drug Trends-B16, K23  
 Drugged Driving-K30  
 Drugs-B77  
 Duchenne Muscular Dystrophy-H97  
 DUID-K27, K28, K29, K31, K43  
 Dumpster Fire-H57  
 Duty to Correct-CB4  
 DWI-K1, K30  
 Dyes-B147

## E

East Asia-A69  
 ECMO-H33  
 Ectopic Cushing's Syndrome-H77  
 EDEWG-J30  
 Edibles-B14, K45  
 EDTA-B32  
 Education-A40, E27, E71, E75, W08  
 EI Fragmentation-B80  
 Elder Abuse and Neglect-E87  
 Elder Abuse Perception-E16  
 Elder Abuse Recognition-E16  
 Elderly-H47, H55  
 Elderly Death Investigation-E87  
 Electric Energy Consumption-D14  
 Electric Energy Meters-D14  
 Electroanalytical Chemistry-B85  
 Electrochemistry-B105  
 Electrocution-H38, H39  
 Electrocution Death-H63  
 Electronic Presentation of Evidence-C18  
 Electronic Signatures-J13  
 Electrothermal Injuries-H39

Elemental Analysis-E3, Y1  
 Eliminating Bias-S01  
 Embolism-H73  
 Embolization-H53  
 EMIT Ecstasy Assay-K10  
 Employment-A40  
 Endangered Wood-E24  
 Endocrine-H125  
 Enforced Disappearances Victims-E27  
 Entomology-E7, H17, H18, H116  
 Environmental Stress-A37  
 EPA vs. NHTSA-D24  
 Epidemic-LW3  
 Epidemiology-H58  
 Epigenetics-F24  
 Epilepsy-H132  
 Equation-A81  
 Error Rate-B108  
 Error Rates-B23, J1  
 Error Tolerance-A79  
 Esophageal Necrosis-H110  
 Estate-J6  
 Ethanol-K1, K30  
 Ethics-A105, A106, A108, F19, LW4  
 Ethics and Policy-I30  
 Ethics Committee-E54  
 European Parliament Directive-E54  
 Evaluation-Y3  
 Evidence-E43, F7, G15  
 Evidence Analysis-E33  
 Evidence-Based Investigation-LW2  
 Evidence Examination-B2  
 Examination-B155, G7  
 Examiner Reliability-B26  
 Excited Delirium-H37  
 Exome Sequencing-H102  
 Exoneration-F1, CB4  
 Experimental Shootings-H135  
 Expert-F7  
 Expert Decision-Making-E78  
 Expert Retention and Selection-J22  
 Expert Software-B137  
 Expert Testimony-F28  
 Expert Witness-B47  
 Expert Witness Credibility-F9  
 Expert Witness Testimony-F8  
 Experts-F5  
 Explosive Analysis-B146  
 Explosive Odorants-B115  
 Explosives-B145, D9  
 Extraction-E9  
 Extremism in Mosque Sermons-D32

## F

Fabric-B87  
 Face Similarity Linkage-I16  
 Facial Identification-C20  
 Fact-Based Opinion-J18  
 Factory Explosion-D5  
 Failures on Electric Energy Meters-D14  
 False Accusations-H3  
 False Evidence-C5  
 False Match Rate-B109  
 False Start-A4  
 Falsification of Crime Scene-E63  
 Familicide-I13  
 Family Mass Murder-I13  
 Farmer's Suicide-E45  
 Fatal-D8, W14  
 Fatal Intoxication-K21  
 Fatal Poisoning-K2  
 Fatality-H66  
 FDE-J11  
 Federated Testing-C8  
 FEM Analysis-D1  
 Femicide-E57  
 Fentanyl-B17, B19, B78, B85, B86, E65, H7, K31  
 Fentanyl Abuse-K21  
 Fentanyl Analogs-B30, B85, K17, K39  
 Fentanyl-Related Compounds-B28  
 Fentanyl Test Strip-B20  
 FES-H70  
 Fibers-B87, B93, B148, E4  
 Field Analysis-B86  
 Field Test-B81  
 Fingerprints-B116, LW1  
 Fire-A62, A63, D8  
 Fire and Explosion-B144  
 Fire Death-A98  
 Fire Debris-B143  
 Fire Debris Analysis-B140, B141  
 Fire Investigation-D10, D11, F27  
 Firearm-B155, H50, Y10  
 Firearm and Toolmark-F30  
 Firearm Identification-B109  
 Firearms-F29, I7  
 Firearms Examination-B108  
 Firefighter Autopsy Protocol-H83  
 Firefighters-H83  
 Fireworks-D5  
 First Molar Length Ratio-G20  
 Flail Mower Injuries-E46  
 Flame Height-D11

- Flash-C31  
Flash Glare-C30  
Flatbed Scanners-J32  
Flood-H35, H123  
Flualprazolam-K48  
Fluctuating Asymmetry-A37  
Fluorescence-A60  
FMVSS 209 S4.4(b)(6)-D20  
Foley Catheter-H72  
“Folie À Deux”-I22  
Footprints-E12  
Force-W14  
Force Plate-J34  
Foreign Terrorist Fighters-E28  
Forensic-B59, E40, E85, H7, H33, H50, H136, H143, K40, K47, W11  
Forensic Analysis-B84  
Forensic Analyst Licensing-F2  
Forensic Anthropology-A1, A3, A5, A9, A14, A16, A29, A32, A35, A39, A42, A43, A44, A45, A50, A54, A59, A64, A70, A72, A76, A82, A92, A93, A97, A99, A104, A109, A110, A111, A115  
Forensic Approach-E42  
Forensic Archaeology-A59, E2  
Forensic Art-E25  
Forensic Autopsy-E69, H32  
Forensic Ballistic-H54  
Forensic Chemistry-A96, B162, K11  
Forensic Computational Linguistics-D30  
Forensic Differentiation of Inks-D22  
Forensic DNA-B49, B137, B139, F26, W02, W19  
Forensic Document Examination-J26  
Forensic Ecology-B132  
Forensic Education-E73  
Forensic Entomology-A95, B27, H12, H15, H21, H22, H113  
Forensic Evaluation-I30  
Forensic Gait Analysis-E11  
Forensic Genealogy-B34  
Forensic Genetic Genealogy-B34, B72  
Forensic Genetic Genealogy (FGG)-B36  
Forensic Genetics-B62, B75, E26  
Forensic Hair Analysis-K26  
Forensic Handwriting-J2  
Forensic Handwriting Examination-J3, J25  
Forensic Imaging-A93  
Forensic Intelligence-B162  
Forensic Investigation-Y8  
Forensic Investigations-Y5  
Forensic Linguistics-J7  
Forensic Medicine-E20  
Forensic Model-I2  
Forensic Multimedia Authentication-W13  
Forensic Odontology-G6, G9, G10, G11, G19  
Forensic Pathology-A82, H61, H64, H69, H75, H76, H78, H90, H111, H134, H138, H141  
Forensic Photography-C30, E36  
Forensic Podiatry-E10, E12, E13  
Forensic Proteomics-B124  
Forensic Psychiatric Assessment-I9  
Forensic Psychiatry-I25  
Forensic Psychology-I21  
Forensic Radiology-E47  
Forensic Science-B39, E8, E38, E44, E61, E62, E87, H25, H74, H127, H135, I15, I17, I18, SC1, W09  
Forensic Science Education-B156, J24  
Forensic Sciences-B67, E17, E63, E66, E67, E69, H23, H46, H47, H57, H60, H68, H88, H89, H93, H105, H126, I20, W04, Y7  
Forensic Serology and Biology-B69  
Forensic Simulation-B144  
Forensic Soil Analysis-B153  
Forensic Stamp Impressions-J34  
Forensic Taphonomy-A65, A73  
Forensic Toxicology-K25  
Forgery-J6  
Formula Sellier-B100  
Foundation-B155  
Foundational Research-J31  
Fourier Transforms-J32  
Fractal Analysis-G10  
Fractography-H140  
Fracture Analysis-A92  
Fracture Healing-A90  
Fracture Patterns-A2  
Fracture Risk Evaluation-D1  
Fragmentary Voltage-B98  
Fraud-W16  
Frequency Tables-A7  
Frontal Sinus-A46  
*Frye*-F30  
FTIR-B67, B161  
Fulminant Myocarditis-H104  
Funduscopy-H4  
Fungal Communities-H20
- G**
- Gait-E11  
Gait Identification-C26  
Garbage Collection-C14  
Garrote-H45  
Gas Chromatography-B146  
Gastric Herniation-H64  
GBL-K24  
GC/MS-B11, B12, B17, B77, Y11  
GEDmatch-W05  
Gellifters®-B25  
Genealogy-B133, CB2, E40, W05  
General Linear Model-B30  
Generative Adversarial Network-C2  
Genetic-E40  
Genetic Genealogy-B35  
Genetically Variant Peptides-B124  
Genetics-H131  
Genotyping-B128  
Geographic Information Systems-A66  
Geographical Locations-H19  
Geolocation-B106, F20  
Geospatial Data-C27  
Geospatial Forensics-C27  
GHB-Y15  
GIS-B149  
Glass-D6  
Glass Evidence-B90  
Glass Standard-D6  
GLScan®-B25  
Glutamate-I28  
Group Contribution Model-D27  
Guidelines-K27  
Gunshot Murder Cases-H54  
Gunshot Passive Defense Injury-H54  
Gunshot Residue-B113  
Gunshot Residue (GSR)-B112  
Gunshot Residues-B102, B111  
Gunshot Residues (GSR)-B105  
Gunshot Trauma Analysis-A1  
Gunshot Wound-H143  
Gunshot Wounds-A9, H52, H134  
Gut-H128
- H**
- H2S-K13  
Hair-K16, K20  
Hair Analysis-E21  
Hair Testing-K7  
Hairs-B59

Hamartoma of Mature Cardiac Myocyte-H82  
 Handguns-B51  
 Hand-Held Raman Spectrometer-B103  
 Hand Sanitizer-H106  
 Handwriting-J1, J27  
 Handwriting Analysis-J8  
 Handwriting Classification-J23  
 Handwriting Examination-J7, J16  
 Handwriting Features-J27  
 Handwriting Variation-J25  
 Hanging-E82, H142  
 Harm Reduction-B160  
 Hashimoto's Thyroiditis-H105  
 Head Injury-D16  
 Head of Ribs-A21  
 Headspace Analysis-B19  
 Health Care Workers-E16  
 Hearsay-I29  
 Heat Release Rate-D10  
 Heavy Truck Crashworthiness-D13  
 Heavy Truck Fire-D12, D13  
 Hemopericardium-H56  
 Hemorrhage-H96  
 Hemostatic Agents-H52  
 Hemothorax-H56  
 Hemp-B11, B12, B13, K46, W17  
 Heroin-B161  
 Heroin-Related Death-K15  
 High Resolution Melt-B71  
 High-Velocity Projectile-E64  
 Hispanic-G18  
 Histology-A71, H30  
 Histomorphometry-A32, A33  
 Histopathology-H88  
 Histoplasmosis-H84  
 HIV-H84, H86  
 Holy Trinity-K32  
 Homemade Explosive-H60  
 Homicide-A7, A100, H48, H144, I10, I14  
 Homicide Firearm Death-E8  
 Hoof Injury-H66  
 Hospital-H74, LW3  
 Houston Cocktail-K32  
 HTML-A49  
 Human Angle-E84  
 Human Biological Variation-A104  
 Human Decomposition-H20, K11  
 Human Factors-E78  
 Human Identification-A106, B69, B75, B106, B122, G2, G6, G9, G11, G13, W15  
 Human Remains-A53

Human Rights-G17  
 Human Variation-A38, A76  
 Humanitarian Forensic-H28  
 Hurricane Harvey-H35  
 Hybridization Capture-B54  
 Hydroxyapatite-G3  
 Hydroxycocaine-K20  
 Hymen-H34  
 Hyoid Fusion-A2  
 Hyperspectral Imaging-D4  
 Hypoxia-H125  
 Hypoxic Damage-H71

## I

Identification-A39, A46, A53, A57, A82, A84, A101, E10, F29, G12, LW1  
 Ignitable Liquid Residue-B142  
 Ignitable Liquid Residues-B99  
 Illicit Drugs-B160  
 Image Analysis-D2, E39, J19  
 Image Processing-A33  
 Immigration Court-II  
 Immunohistochemistry-H104, H142, K15  
 Immunostaining-H77  
 Impact Mechanism-A92  
 Implementation-CB1, F4, SC1, W07  
 Improvised Explosive Device-E28  
 Impulsive Aggression-I28  
 In-Air-J15  
 Inconspicuous Features-J18  
 In-Custody Death-H75  
 Indanedione-B94, E37  
 Indigenous Perspectives-Y2  
 Individualistic Characteristics-E13  
 Ineffective Prosecution-D24  
 Infant-A87  
 Infant Death-H8  
 Infant Homicide-E68  
 Infectious Diseases-H85  
 Infectious Esophagitis-H76  
 Inflammatory Cardiomyopathy-H104  
 Infrared Photography-E19  
 Infrared Spectroscopy-B91  
 Inhalant-E85  
 Inhalants-H107  
 Inhibition-B76  
 In-House Counsel-F3  
 Injuries-H44  
 Injury-D15, E14  
 Injury Mapping-C18  
 Injury Pattern-E48, H48

Ink Analysis-J19, J33, CB5  
 Innocence-F18  
 Innocence Project-E74  
 Innovation-E76  
 InnoXtract™-B127  
 Insanity Defense-I17, I18  
 Insect Succession-B154  
 Insertion Deletion Polymorphisms-B121  
 Intermediate Target-H51  
 Intermittent Explosive Disorder-I23  
 International-B123, E75  
 Interpretation-B111, B138  
 Interpretation of Tire Marks-D18  
 Intestinal Perforation-H91  
 Intoxication-H109, K7, K13  
 Intra-Abdominal Pressure-H98  
 Intracranial-H50  
 Intraoral Scanner-G13  
 Intraosseous Puncture-H99  
 Investigation-B66, E85  
 Investigative Genetic Genealogy-B34  
 Investigative Information-G15  
 Investigative Leads-B55  
 Investigative Practices-Y2  
 Investigative Public Service-E74  
 Invisible Ink-J21  
 IOS®-C25  
 IoT-C6  
 Irish-LW3  
 Ischemia-H125  
 ISO-C30  
 Isolation-E41  
 Isopropanol-H10  
 Isoscaping-A57  
 Isothermal Amplification-B1  
 Isotonitazene-K5  
 Isotope Analysis-A55, A56, B106  
 Isotopes-A57  
 Italian Data-E57

## J

Jeju Island-B64  
 Jigsaw-H59  
 Judge-F15  
 Junk Science-F25  
 Jurisprudence-F23, W09  
 Jurors and Science-F6  
 Justice-E43  
 JUUL®-K24  
 Juvenile-A19, B125  
 Juvenile/Adult Legal System-G17

## K

Kanesaki Micki Case-E17  
 Kavain-K49  
 Kennedy-B107  
 Kinship-B123  
 Kinship Inference-B42  
 Knives-D23  
 Knowledge Management-C28  
 Knowledge Production-A102  
 Known Source Collection-B116  
 Kuwaiti Reference Data Set-G16

## L

Laboratory Counsel-F3  
 Laboratory Lawyer-F3  
 Laboratory Testing-B8  
 Lacquered Cartridges-E6  
 Landmarks-A51  
 Language-A103  
 Laparoscopic Surgery-H40  
 Lapidation-E59  
 Laser Ablation-B102  
 Last Words-D29  
 Latent Blood Detection-B50  
 Latent Fingerprints-E35  
 Latent Print Friction Ridges-E37  
 Latent Prints-B24, B25, B92, B94, B117  
 Latent Prints Labs-B23  
 Latent Touch Samples-B157  
 Latinx-A80  
 Law Enforcement-E18, H37  
 Law School-University Collaboration-F11  
 Laws-J26  
 Lawyer-F15  
 LC/MS/MS-K5, K16, K20, K42, K44  
 LC-qTOF-K17  
 Lead-Free Ammunition-B102  
 Learning Algorithms-B90  
 Legalization-K43  
 Leukostasis-H79  
 LIBS-A54, B101, B105  
 LiDAR-F21  
 Lies-D33  
 Ligature Mark-E50  
 Light Detection And Ranging (LiDAR)-  
 C32  
 Likelihood Ratio-B41  
 Likelihood Ratios-B42  
 Limit of Detection-E1  
 Linguistic Markers-D32

Linguistics-D31  
 Lip Prints-F25  
 Lipsticks-E4  
 Liquid Chromatography-D3  
 Liver Hematoma-H95  
 Lividity-H34  
 Living Victim-H145  
 Living With the Dead-E41  
 Logic-G7  
 LSD-K34  
*Lucilia Sericata*-H13  
 Lumbar Vertebrae-H139  
 Lysozyme-B3

## M

Mac<sup>®</sup> Operating System-C4  
 Machine Indentations-J28  
 Machine Learning-A96, B141  
 Mackworth Clock Test-H89  
 Macro Development-J31  
 Magnetic Flux-J29  
 Magnetic Resonance Imaging-A22, A78  
 Maltreatment-E67  
 Management-E79, K4  
 Management of the Dead-E84  
 Mandible-G9  
 Manner of Death-H39, I8  
 Manual-H41  
 Marijuana-B11, B12, B13, B81, W17  
 Marine-A94  
 Marketing-J22  
 Mass Disasters-H36  
 Mass Graves-A58  
 Mass Spectrometry-B14, B30, B83, E24,  
 K24, W07  
 Massively Parallel Sequencing-B132,  
 B134  
 Material Characterization-D22  
 Matricide-I14  
 Mattress Fires-A64  
 Maxillary Sinus-A30  
 McKern-Stewart Method-A25  
 MDPV-K37  
 Mechanical Asphyxia-H123  
 Mechanical Restraints-H74  
 Medial Clavicle-A24  
 Medial Clavicular Epiphysis-A23  
 Mediastinal Neoplasm-H1  
 Medicolegal-E88, H18  
 Medicolegal Death Investigation-H29  
 Medicolegal Death Investigator-E44  
 Medicolegal Investigation-H48  
 Medullary Thyroid Carcinoma-H77  
 Memory Forensics-C3, C4  
 Mental Health-I3  
 Mental Health Issues-I29  
 Mental Illness-I25  
 Metabolism-K38  
 Metabolite-B4  
 Metabolomics-A96, B5  
 Metadata-C1, C11  
 Methamphetamine-H8  
 Methanol-H106  
 Methemoglobin-K6  
 Method Development-B157  
 Method Evaluation-A77  
 Method of Suicide-H58  
 Method Validation-A55, A56  
 Methodological Approach-F10  
 Methods-A83  
 Methylphenidate-K42  
 Metric Analysis-A48, A79  
 Metric Sex Estimation-A44  
 Microbial DNA-H129  
 Microbial Ecology-H114  
 Microbial Succession-H14  
 Microbiome-B70, B131, H13, H19, Y14  
 Micro-CT-A31, H138  
 Micro-CT Scans-A107  
 Microhaplotypes-B42, B63  
 Micromanipulation-B135  
 Micro-Measurement-E5  
 Microscopy-B91, D17  
 Microwaves-B48  
 Migrants-A105, E15  
 Migration-A37  
 Military-E28  
 Mimic-H99  
 Mineralized Tissue Histology-G4  
 Minerals-B89, B153  
 Miniaturized UV/Vis-J33  
 Minimally Destructive-J33  
 Misconduct-F2  
 Missing-E29  
 Missing Person-B123, H68  
 Missing Persons-E30, I2, Y6  
 Missing Teeth-G2  
 Mitochondrial DNA-B133  
 Mitochondrial DNA Haplogroup-B64  
 Mitochondrial Genomes-B54  
 Mixed Samples-B45  
 Mixed Traces-B137  
 Mixture-B43



Mixture Interpretation-B37, B129  
Mixture Simulations-B136  
Mixtures-B135  
MMIW Crisis-A100  
MMIWG-Y2  
Mobile Device Forensics-C12, F22  
Mobile Forensics-C8, C13  
Modeling-A79  
Modifications-D15  
Molecular Autopsy-H102  
Molecular Testing-H132  
Monitoring Testimony-B118  
Morphological Evaluation-A30  
Morphometrics-A13, A16  
MorphoPASSE-A13  
Motor Vehicle Accident-H64  
Movement Prediction-C26  
MRM-B6  
MtDNA-H131  
Multicenter Trauma Registry-A89  
Multidisciplinary Approach-H59, H126  
Multidisciplinary Investigations-D5  
Multimedia-C29, W03  
Multimedia Forensics-C2  
Multiple Sampling-H120  
Multivariate Analysis-B80  
Multivariate Data Analyses-B63  
Mumbai City-H28  
Mummified-E41  
Murder-E52, E63, H46  
Murder and the Military-I24  
Muscle-H118  
Muzzle-to-Target Distance-B110  
M-Vac<sup>®</sup>-B53, B73  
Myocarditis-H30

## N

NamUs-A99, E30, H121  
Nanobiosensor-B3  
Nasopharyngeal Swabs-H27  
Natural Language Engineering-D31  
Neck Injury-H49  
Neck Stab Wound-E60  
Necrobiome-H15  
Needle Exchange-B21  
Network Analysis-C6  
Neural Network-J23  
Neurocognitive Testing-I27  
Neuropathology-H73  
Neurotoxin-H11  
Neutrophils-H31

Next Generation Sequencing-B46, B59,  
B130, B131, B133  
NFLIS-B16  
NFPA 921-F27  
NGS-B44, B129  
N-Hexane Intoxication-E86  
NHTSA-D21  
NHTSA Crime-D19  
NIST SRM 2372a-B60  
Nitrogen Inhalation-I12  
Nitrous Oxide-H71, H107  
NMR Spectroscopy-B79  
Non-Targeted Methods-S01  
NORSE-H102  
NovaQUANT<sup>™</sup> QPCR-B60  
Novel Psychoactive Substances-K47  
Novel Synthetic Opioid-K38  
NPS-K34, K36, W11  
NPS Surveillance-W06  
Number of Contributors-B139

## O

Objective-C-C4  
Obstructive Sleep Apnea-G5  
Occupational Injuries-E64  
Odontometrics-A80  
Officer Involved-E19  
Officer-Involved Shootings-E33  
Olfactometer-B115  
Omission-D33  
On-Duty Death-H83  
Online-E77  
Online Instruction-B156  
Online Learning-E73  
Online Teaching Program-E27  
Open Source-A58  
Operating Procedures-Y10  
Opinion Measures-J3  
Opioid Abuse-K52  
Opioid Detection-B19  
Opioid Use-A34  
Opioids-B21, W04  
Opium-B84  
O-PTIR-B151  
Orbital Area-A10  
OSAC-B29, B152, CB1, F4  
Osteometrics-A52  
Osteometry-A62  
OTC Drugs-K50  
Otorrhagia-H45  
Outdoor Research-A6

Oversight Regulation-F2  
Oxycodone-K41, K52  
Oxymorphone Glucuronide-K41

## P

Pack Rat Nests-A65  
Paint-B151  
Palatal Rugae-G13  
Palynology-B150  
Pandemic-LW2  
Paper Examinations-J28  
Paper Textures-J32  
Papillary-H94  
Paralytic Shellfish Poisoning-H11  
Parental Behavior-I11  
Partition Coefficient-D27  
Patch Inhalation-K21  
Patella-A18, A61  
Pathological Impulsiveness-I24  
Pathological Impulsivity-I23  
Pathology-C18, H136  
Pattern Analysis-E31  
Patterned Injury-H51  
PCAST-F5  
PCP-K3  
PDF-J17  
PDMAC-B92  
Pedagogy-W08  
Pediatric-A3, A88, H101, K50, K53  
Pediatric Death-H7, H97  
Pediatric Skeletal Trauma-A89  
Pediatric Sudden Unexpected Death-H1  
PE-H81  
Pen Pressure-J5  
Penetrating Chest Injury-E64  
Pepper Spray-B104  
Performance Assessment-B26  
Performance Validation-B140, B141  
Performances-B23  
Periodontal Ligament-G14  
Per Se-K28  
Persistence-B125  
Persistent Organic Pollutants-Y11  
Personal Data Access-C6  
Personality Disorder-I24  
Personality Disorders-I10, I14  
Pesticide-K8  
Petechiae-H41  
Petroleum Ether-B161  
Pharmacodynamics-K37  
Pharmacokinetics-K37, K41

Phone Screen-E55  
 Photogrammetry-C21, C22, C32, G2  
 Photography-C31  
 Physical Fit-B95  
 Pitfall-B43  
 Placenta-H96  
 Plantar Pressure Distribution-E12  
 Plastics-E5  
 PMCMR-E86  
 PMCT-E86, H122  
 PMI-H115, H118, H119, H130  
 PMI Delimitation-H120  
 Point Cloud-F21  
 Points of Concordance-G1  
 Police-I3, W14  
 Police Violence-A116  
 Policy-A86  
 Pollen-B150  
 Polychlorinated Biphenyls (PCBs)-I27  
 Polydrug-E65  
 Polymers-B114  
 Polypharmacy-K31  
 Population Affinity-A114  
 Population History-A80  
 Population Specificity-A77  
 Population Study-B91, B148  
 Pop-up-C31  
 Porn-C7  
 Porous Surfaces-B94  
 Portable Instrumentation-B86  
 Positive Identification-G1  
 Post-Blast-D9  
 Post-Conviction-F11  
 Posterior Head-H143  
 Postmortem-G1, K23, K47, K48, K50, K53  
 Postmortem Blood-K39  
 Postmortem Computed Tomography-H141  
 Postmortem CT-H80, H139  
 Postmortem Diagnosis-K15  
 Postmortem Findings-H2  
 Postmortem Interval-A74, E39, H12, H17, H117  
 Postmortem Interval Estimation-A66, A72  
 Postmortem Microbiome-H127, H129  
 Postmortem Submersion Interval-D4, H14  
 Postmortem Tooth Loss-G14  
 Postmortem Toxicology-K51  
 Potency-K36  
 Predictive Fire Models-D11  
 Predictive Identification Method-B28  
 Pre-Existing Medical Conditions-H24

Pregnancy-H95, H96  
 Pressure Distribution-D1  
 Presumptive Testing-B50, B52  
 Prevention-E68  
 Printing-W16  
 Prison-I20  
 Probabilistic Genotyping-B37, B38, B39, B40, B51, B129, W02  
 Probabilistic Language-H3  
 Process State-C14  
 Proficiency-K9  
 Proficiency Testing-E80  
 Profiling-B113, I6  
 Projectile Energy-B87  
 Projectile Trauma-A8  
 Protein-H119  
 Protein Degradation-H115, H118, H120  
 Proteomic Genotyping-B124  
 Proteomics-B6  
 Psychiatric-W18  
 Psychiatrist-I4  
 Psychiatry-I15  
 Psychology-I19, Y12  
 Psychopathy-I18  
 Psychopathy and Perversion-I26  
 Psychosis-H37  
 Psychotic Decompensation-I21  
 PTSD-I5  
 PTSD-C-I5  
 Puberty-A19  
 Pubic Symphysis-A27  
 Public Policy-I3  
 Publications-A41  
 PUD-H81  
 Pull-Up-B126  
 Punching-E20  
 Pyrolysis GC/MS-D22  
 Pyrolysis Products-B99

## Q

QRM-B159  
 Quality-K9  
 Quality Assurance (QA)-A55, A56  
 Quality Control-A45  
 Quality Metrics-B24  
 Quantification-D23  
 Quantitation-B57, K40  
 Quantitative Forensic Linguistics-D29  
 Quantitative PCR-Y14  
 Quantitative Reliability Metric-B159  
 Questioned Documents-J9, J13, J19, J29

## R

R.I.P.<sup>®</sup>-H53  
 Race-A35, A103, A109, A110, A113, A114  
 Racecraft-A116  
 Racial Bias-Y6  
 Racial Disparities-F19  
 Racial Identity-A112  
 Racial Violence-A100  
 Racism-A116  
 Radiocarbon Dating-A70  
 Radiographs-A47  
 Radiology-A46  
 Raman-B151  
 Raman Microscopy-B88, B153  
 Raman Spectroscopy-B15, B89, B114, B147, E38  
 Random Forest Modeling-A52  
 Random Forests-J20  
 Randomly Acquired Characteristics-B26  
 Range of Certainty-C22  
 Rape-B47, B68  
 Rapid-B48  
 Rapid DNA-B66, F16, F17, Y8  
 Rapid DNA Analysis-B55  
 Rapid DNA Technology-B75  
 Rapid Drug Screening-B82  
 Rapid Transmission-C24  
 Rare Etiology-H76  
 Rational Capacity-I17  
 RED-BLEU-B32  
 Re-Examination-E14  
 Reference Collection-J9  
 Regional-B58  
 Regression Analysis-G20  
 Relevant Scientific Community-F30  
 Reliability-A43  
 Reliability and Validity-J3  
 Religious Extremism in Pakistan-D32  
 Religious Setting-I9  
 Remediation-B112  
 Remote Instruction-W08  
 Reproducibility-B9  
 Research-E76  
 Residual Odor-B97  
 Respiratory Failure-H97  
 Restraint-H47  
 Retinal Air Emboli-H4  
 Retinal Hemorrhages-H103  
 Retropharyngeal Abscess-H49  
 Retrospective Review-H26

Retrospective Study-I8  
Review-H119  
Rib Fracture-A88  
Rib Fractures-A87  
Risk-C7  
Risk Factors-E68, H81  
Roadside Tire Marks-D18  
Rodenticides-K7  
Rootless Hair-B35  
Rootless Hair DNA-B127  
Rules of Evidence-F5

## S

Saccular Aneurysm-H101  
Sacrum-A15  
Sailfish OS-C9  
Saliva Identification-B3  
*Salmonella Enterica*-H13  
Sample Collection-B73  
Sampling-B2  
SARS-CoV-2-H25  
Saxitoxin-H11  
Scanning Electron Microscopy-H67  
Scattering Patterns-A67  
Scavenging-A67  
Scene Documentation-E2  
Scene Reconstruction-E33  
Scene Recovery-A65  
Scientific Evidence-F10  
Scientific Literature-W19  
Score Likelihood Ratios-B90  
Screening-B17, B18, K44  
Screening Method-K14  
SDO-B152  
Searches-I2  
Secondary Prevention-E49  
Secondary Projectile-H51  
Secondary Transfer-F8  
Seized Drug Analysis-B82  
Seized Drugs-B18, B22, B80  
Seized Samples-D3, D28  
Self-Efficacy-E53  
Self-Immolation Suicide-H57  
Self-Inflicted-E81, H56  
Self-Inflicted Gunshot Wounds-H61  
Self-Strangulation-Y4  
Semen-H116  
Semen Identification-B52  
Semen Marker-B68  
Seminal Stains-B61  
Seminal Vesicle-Specific Antigen-B68  
Sepsis-H31  
Sequencing-W15  
Serial-E62  
Serial Killer-I16  
Serial Numbers-B114  
Serology-B10, E71  
Severe Mental Disorder-I22  
Sex Determination-A11  
Sex Estimation-A10, A12, A13, A14, A15, A16, A17, A18, A19, A36, A101  
Sexual Abuse-H34  
Sexual Assault-B33, B70, B125, K33  
Sexual Assault Samples-B10  
Sexual Dimorphism-A14  
Sexual Perpetrator-I26  
Sexual Violence-E26  
Shaken Baby Syndrome-H5  
Sharp Force Trauma-A5, E56, H65  
Sharpness-D23  
Shell Casings-B7  
Shootings-Y12  
Short Survival-H42  
Short Tandem Repeats-B46  
Shotgun Injuries-B100  
Signature-J6  
Signature Examinations-J5  
Signature Identification-J24  
Signatures-J2, J12, J14, J15, W01  
Silicone-H73  
Similarity Scores-B95  
Simulation-D8, Y7  
Singapore-B148  
Single Cell-B45, B135  
Single Nucleotide Polymorphism-B36  
Single Nucleotide Polymorphisms-B130  
Single-Cell Forensic Analysis-B49  
Skeletal Age Assessment-A20  
Skeletal Collection-A6  
Skeletal Development-A77  
Skeletal Population-A112  
Skeletal Reconstruction-A50  
Skeletal Remains-Y3  
Skeletal Tissues-K12  
Skeletal Trauma-A6  
Skin Color-E23  
Skin/Sweat-B71  
Slash Injuries-H46  
SNP Genotyping-B72  
SNPs-CB2, W05  
Social Implications-F19  
Social Science-A86  
Socio-Economic Status-A38  
Sodium Nitrite-H111  
Sodium Nitrite/Sodium Nitrate-K6  
Software-B57, E61  
Software Validation-B40, W03  
Soil-B97  
Soil Chemistry-H20, H114  
Soil Color-B149, B152  
Soil Survey-B149  
Solid-Phase Extraction-B145  
Solid-Phase Microextraction-B77  
Solvatochromic/Vapochromic-E34  
Source-F14  
South Africa-A67, E15, K18  
Sparse Chemometrics-E4  
Speaker Recognition-C19  
Special Session-S02  
Species-A52  
Species Identification-E24  
Speech-Generating Devices-F9  
SpermX™-Y9  
Spine Surgery-H70  
SPME-GC/MS-B78  
SQLite-C25  
SQLite Data Recovery-C8  
Stab Injuries-A5  
Stab Wound-Y5  
Stab Wounds-E66  
Stability-K39  
Staffing-E79, K4  
Staged Crime Scene-H144  
Stalking Law-E57  
Stamp Clone-J35  
Stamping Devices-J34, J35  
Standardization in Reducing Bias-S01  
Standards-CB1, C19, C20, D25, D26, J11, SC1, W09  
Statistical Analysis-A4  
Statistical Design of Experiments-K26  
Statistical Probability of Match-B159  
Statistics-J20, J27  
Stature Estimation-A47, A81  
Steganalysis-C17  
Steganography-J21  
Stillbirth-E69  
Stimulant-B20  
Stir-Bar Sorptive Extraction-Y11  
STK® Sperm Tracker-B52  
STR Analysis-B40  
STR Profile-Y9  
STR Profiling-B65  
Strangulation-E50, H41, H44, H45

STRmix™-B37  
 STRs-B120  
 Struck by a Car-E58  
 Stutter-B39  
 Subacute Combined Degeneration-H107  
 Subadult Skeletal Remains-A51  
 Subarachnoid Hemorrhage-H101  
 Subdural Hematoma-H84  
 Sublimation-LW4  
 Submergence-H21  
 Substance Abuse-K19  
 Substrates-B99  
 Subtropic Region-A68  
 Suchey-Brooks-A26  
 Sudden and Unexpected Death-E49  
 Sudden Cardiac Death-H87, H90, H92  
 Sudden Cardiac Death (SCD)-H93  
 Sudden Death-H79, H94, H132  
 Suicide-E47, E56, E60, E66, E81, H6,  
 H29, H55, H58, H59, H61, H108, I8,  
 K2, K6, K19, K35  
 Suicide Firearm Deaths-E8  
 Suicide Note-H60  
 Suicide Notes-D29  
 Surface Effect-B5  
 Survey-Y15  
 Survival Intervals-H134  
 Swab-Y14  
 Syringes-B21  
 Systematic Comparison-B95

## T

TA3-A28  
 Takayasu Arteritis-H94  
 Tandem Mass Spectrometry-B28, K12  
 Tape-E9  
 Tape Evidence-E35  
 Tape Lifts-B9  
 Taphonomic Alterations-A68  
 Taphonomy-A50, A69, A72  
 Tarawa-A8  
 Target Factor Analysis-B143  
 Taser®-E19  
 Tattoo-E36  
*Taxus Baccata*-K2  
 TBS-H16  
 T-Cell Lymphoblastic Lymphoma-H1  
 Teamwork-E42  
 Tech Effect-F6  
 Technical Error of Measurement-A62  
 Techniques-G12

Technology-E61, J10  
 Telehealth-I30  
 Tele-Testimony-I29  
 Temperature-H115  
 Terrestrial LiDAR Scanning-E22  
 Testimony-F26, I4, J26, W10  
 Testimony Transcript Review-B118  
 TETRYL-B98  
 Texas-F4  
 Text Analysis-A41  
 Text Monitor-E55  
 Textile Damage-H113  
 Textile Dye-B88  
 Textiles-B147, E39  
 Thanatobiota-H128  
 THC-K43, K45  
 Thermal Alteration-A61  
 Thermal Damage-A98  
 Thermal Paper-B92  
 Thickness-E5  
 Thin-Layer Chromatography-CB5  
 Thiofentanyl-K14  
 Thiosulphate-K13  
 Third Molar-G19, G22  
 Thoroughness-G7  
 Threat Agnostic-W06  
 Thromboembolism-H88  
 Throttling-E17  
 Time of Colonization-H17  
 Time Since Death-A70, G14  
 Time Since Injury-A90  
 Tire Marks-D18  
 Tires-E3  
 Tissue Procurement-H10  
 TLC-CB5  
 Toner-J29  
 Toolmark-A4, Y10  
 Toolmarks-E6, F29  
 Tools-C25  
 Tooth Anatomy-G8  
 Tooth Dimensions-G11  
 Tooth Root Translucency-G3  
 Torsion and Compression-E48  
 Touch DNA-B43, B74, B131  
 Touch Sample-B7  
 Toxic Exposure-I27  
 Toxicity-B22, K8  
 Toxicological Exam-E51  
 Toxicological Investigation-H43  
 Toxicology-E65, H8, H9, K3, K4, K18,  
 K19, K33, K38, K40, K49, K53, W11  
 Trabecular Analysis-A31

Trace-B56  
 Trace Analysis-B145  
 Trace Elements-D6  
 Trace Evidence-B33, B93, B132, E3  
 Trace Materials-B29  
 Tracheoesophageal Fistula-H86  
 Tractor Runover-E45  
 Tractor-Related Death-E45  
 Traffic Accidents-G5, H62  
 Training-E76  
 Training Standards-W19  
 Tramadol-K12  
 Transfer-F7, W10  
 Transfer DNA-B51  
 Transgender-A101  
 Transition Analysis-A27, A28  
 Transparency-F13  
 Transvestism-E82  
 Trash Marks-J31  
 Trauma-A83, E88, I1  
 Trauma Analysis-A7, A91  
 Trauma in Neck-A2  
 Traumatic Axonal Injury-H42  
 Traumatic Death-H122  
 Traumatic Deaths-E42  
 Trazodone-I12  
 Trends-K22, K29, K34  
 Trial-LW2  
 Trocar-H40  
 Truck Fuel System Defects-D13  
 Tuberculosis-H86, H91  
 Tucson Sector-A74

## U

UAS Forensics Framework-C16  
 Umbilical Cord-K25  
 Uncertainty-K9  
 Unclaimed Bodies-A84  
 Underwater-A60  
 Unexpected Death-H70  
 Unidentified-E15, E29  
 Unidentified Bodies-A84  
 Unidentified Human Remains-G3  
 Unidentified Migrants-A17  
 Unidentified Remains-H121, Y6  
 Unmanned Aircraft-C16  
 Untargeted Analysis-W06  
 Untruth-E43  
 Unusual Lesion Pattern-E46  
 Upholstered Furniture-D10  
 UPLC/MS/MS-K14





Urethral Injury-H72  
Urethral Laceration-H72  
Used Syringes Analysis-B160  
Userland Memory Forensics-C14  
Utility-A24  
UV-Vis Microspectrophotometry-B93  
Uxoricide-I25

## V

---

Vacuum-B74  
Vacuum Dispersion-B89  
Vacuum Ultraviolet Spectroscopy-B146  
Validation-A42, B57, B120, B139, K17  
Vapor Delivery-E1  
Variation-J2  
Vascular Dysfunction-H63  
Vascular Injury-H40  
Vasculitis-H90  
Venous Thromboembolism-E49  
Vertebral Canal-A38  
Veterinary-H136  
Veterinary Forensic Pathology-H137  
*Vibrio Vulnificus*-H78  
Vicarious Trauma-I5  
Video Acquisition-C15  
Video Analysis-C21  
Video Authentication-C1  
Video Comparison-E11  
Video Conferencing-C1  
Video Forensics-C15  
Video/Imaging Technology & Analysis-C20  
Virome-B122  
Virtopsy-H123  
Virtual-E77  
Virtual Anthropology-A49  
Virtual Curation-A48  
Virtual Lab-E71  
Virtual Laboratories-B156  
Virtual Reality-E70, F12  
Virtual Tours-E73  
Virtual Trauma Assessment-A93  
Visualization-C3  
Vitality-H142  
VOC-H16  
VOC Cross-Contamination-E34  
Vocabulary-B119  
VOCs-B78

Voice Recorder-C10  
Volatile Organic Compounds-B96, B97, B154  
Volatiles-H117  
Vulnerable Communities-A106  
Vulture Scavenging-A66

## W

---

Warning Signs-I13  
Washed Semen Stains-B69  
Watermarks-J28  
Wavelet Analysis-B103  
Wearer DNA-B73  
Web-Based Forums-K35  
Webbing Breaking Strength-D20  
WEIRD-A102  
Well-Being-I7  
Wet-Vacuum-B53  
WGS-B35  
WhatsApp-C29  
Wild Animal Fatalities-H65  
Wildlife Forensics-B54  
Wischnewsky Spots-H110  
Wood Substrates-B110  
Workforce-E79  
Workplace Drug Testing-K46  
Workplace Safety-E44  
Workplace Stress-E78  
Work-Related Death-E46, E48  
World War II-A8  
Wound Infection-H78  
Writer Identification-J18  
Writer's Cramp-J25  
Wrongful Conviction-F22

## Y

---

YFSF-S02  
Young Forensic Scientists Forum-S02  
Youth Gangs-E18  
Youth Violence-E18  
Y-STR-B64

## Z

---

Zebrafish-H138  
Zonation-A51  
Zooarchaeology-A53



# Presenting Author Index—2021

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2021 Annual Scientific Meeting. The reference table below assists you in finding the section in which the presentation will be given. Letters correspond to the scientific discipline/section in which the presentation is being made while the number corresponds to the numerical sequence of the presentation within the section.

A	Anthropology	I	Psychiatry & Behavioral Science
B	Criminalistics	J	Questioned Documents
C	Digital & Multimedia Sciences	K	Toxicology
D	Engineering & Applied Sciences	LW	Last Word Society
E	General	CB	Case Break Session
F	Jurisprudence	S	Special Session
G	Odontology	SC	Standards Consortium
H	Pathology/Biology	W	Workshop

## A

Acharya, Ashith B. - G20  
Adamowicz, Michael S. - B122  
Adcox, Devin L. - A48  
Adelizzi, Betsy - CB3  
Ademaj, Xhemajl - B98  
Adeoye, Alexandra - B30  
Adkins, Michael T. - J21  
Aggazzotti, Cristina - D29  
Ahmed, Dana A. - G19  
Akiyama, Cliff - E18  
Ali-Gombe, Aisha - C3  
Alkandiri, Fatemah - G16  
Almazrouei, Mohammed A. - E78  
Almela, Angela - D30  
Almirall, Jose R. - S01  
Alsaif, Dalia M. - H34  
Al-Sarraj, Safa - H42  
Alsop, Kirsty - A4  
Ambrosious, Teresa - CB1  
Amuso, Rebecca - E68  
Anderson, Joe P. - C30  
Andreaggi, Kimberly S. - W15  
Andronowski, Janna M. - A34  
Annunziata Nicolaidis, Kathleen - J13  
Appleford, Colin - H78  
Appley, Meghan - E24  
Aquila, Isabella - E17, E61, E62, E63,  
E66, E67, E69, H23, H46, H47, H60,  
H68, H74, H88, H89, H93, H105, I15,  
I20, Y7  
Aquila, Valerio R. - H89, I15  
Armstrong, Melanie Thomas - E40  
Armstrong Hoskowitz, Natalie - W18  
Asen, Daniel - LW1  
Atasoy, Sevil - J24  
Ather, Ferdows Z. - I29  
Avellaneda, Lucio - B121  
Ayers, Richard - C8, C23

## B

Badson, Jeovanna S. - Y15  
Bailey, Calli - F1  
Baker, Susan J. - G14  
Baldari, Benedetta - E56, H59  
Baldino, Gennaro - D5, Y5  
Ballew, Alfarena - E65  
Banks, Petra - A63  
Barco, Lesly - B94  
Barker, Julia M. - CB5  
Barnhart, Tylor - B66  
Baronti, Arianna - E45  
Barranco, Rosario - H45  
Basic, Zeljana - A10  
Battiato, Sebastiano - C2  
Bayer, Raymond H., III - Y13  
Beales, Elsie - D4  
Beardsley, Sara - H97  
Beauchamp, Alain - B109  
Behan, Michelle L. - F25  
Behnke, Grayce - K1  
Behonick, George S. - K49  
Belganeh, Rojin - D22  
Belli, Giacomo - I8  
Belova, Natalia - H56  
Benecke, Mark - B47  
Benevento, Marcello - Y4  
Bernard, Melissa - Y1  
Bernstein, Kadie F. - H19  
Bertozzi, Giuseppe - E49, E50, E51, E52,  
E57, F10, H59, H71  
Bertrand, Benoit - A70  
Bethard, Jonathan D. - Y6  
Bhaloo, Zain - S02  
Bieber, Frederick R. - W15  
Bierly, Jolene - K29  
Bloom, Joseph D. - I18  
Blum, Marc-Michael - W06  
Bolhofner, Katelyn L. - A2

Bonaccorso, Luana - I11  
Bonetta, Carlo Filippo - E61, E62, H46,  
H60, H105, I20, Y7  
Bonner, Miquellie B. - B88  
Border, Samantha - B128  
Borrini, Matteo - A9, A50, LW2  
Bosch, David R. - D12, D13, D21  
Bosco, Caterina - I11, I14  
Bostick, Desmond R. - C31  
Bottari, Giampiero - H54  
Bou Nassif, Jose - I28  
Boulnemour, Keenan - H50  
Bozenko, Joe - B31  
Bracewell, Nicole - K19  
Bradley, Lucy - H4  
Braun, Madeleine - B101  
Brenner, Charles H. - B43  
Brissette, Elizabeth M. - I19  
Brixen, Eddy B. - C5  
Brooks, Emily - A16  
Brooks, Jared - H143  
Brown, Catherine O. - S02  
Brown, RYanne - Kase Break Se34  
Brown, Theodore T. - H25  
Bruehs, Walter - C15  
Brunty, Josh - C12  
Bucht, Rebecca E. - B8, W10  
Buckingham, Christian - K12  
Buckley, Josephine - B40  
Budmark, Amber D. - K14  
Budowle, Bruce - W02  
Bugajski, Kristi - H22  
Bügler, Jürgen - J30  
Bunch, Ann W. - E30  
Buongiorno, Luigi - B100  
Burns, Amber - W07  
Butler, Erin R. - B6  
Butler, John M. - W19  
Buzzini, Patrick - W02

## C

Cacciatore, Giulia - E61, E62, E67, E69, H46, H60, I20  
 Cale, Cynthia - B51, B55  
 Campagna, Shawn R. - A96  
 Campbell, Kirk - W05  
 Canale, Lauren C. - B59  
 Candela, Kylie E. - K51  
 Cantor, Erica - A76  
 Capitaneanu, Cezar - G17  
 Caputo, Fiorella - E61, E62, H46, H88, H93, I20  
 Carelli, Claudia - K7  
 Carew, Rachael M. - J35  
 Carlotti, Jessica - B142  
 Carney, Esq., John J. - F22  
 Carroll, Marla E. - C20  
 Casey, Eoghan - C28  
 Cassata, Griffin - B81  
 Castellani, Rudy J. - H5  
 Cavus, Oktay - I7  
 Cawley, Hannah M. - Y8  
 Chamberlain, Michael - F5  
 Chambers, Megan I. - B14  
 Champod, Christophe - B116  
 Chany, Christopher P. - B112  
 Chapman, Brendan - B70, E55  
 Chaski, Carole E. - D31  
 Chaudhary, Muhammad Taimoor - B84  
 Chen, Alan W. - I30  
 Chen, Xiao - B9  
 Cheng, Kevin - B129  
 Cheng, Ya-Chih - B11, B134  
 Cheong, Harin - H77  
 Chesson, Lesley A. - A56  
 Chew, Roland G. - G1, G8  
 Cho, Helen - A109  
 Cho, Sohee - B64  
 Choudhuri, Satyam - I4  
 Christensen, Erica R. - A46  
 Chu, Sarah - F19  
 L. Scott Chumbley - B108  
 Cinco, Roehl Marave - B161  
 Ciottoni, Gregory - W06  
 Cipolloni, Luigi - E49, E50, E51, E52, E56, E59, E60, H59, H71  
 Ciruzzi, Maria S. - I26  
 Clark, Brian S. - S02  
 Clay, Michael - G4  
 Clermont-Beaudoin, Valerie - B126  
 Coble, Michael D. - W02

Cochrane, Katherine - H121  
 Cody, Robert B. - W07  
 Cole, Mary E. - A33  
 Cole, Stephanie J. - A19  
 Collings, Amber - W08  
 Concheiro-Guisan, Marta - K37  
 Congram, Derek - A108  
 Connon, Catherine Cupples - E71  
 Cop, Maja Jelena - K10  
 Corbally, Michelle - B143  
 Cordasco, Fabrizio - E61, E62, E69, H46, H47, H60, H68, H74, H88, H93, H105, I15, I20  
 Coreelman, Heleen - A78  
 Corron, Louise K. - A38  
 Corsaletti, Linda - I6  
 Cosgriff-Hernandez, Meghan-Tomasita J. - A111  
 Costantino, Andrea - E47, E48  
 Cotton, Robin W. - W19  
 Court, Michael - W06  
 Cox, Nancy - J2  
 Coxon, Keely - H84  
 Crespo Cajigas, Janet M. - E34  
 Croom, Nicole A. - H102  
 Cropper, Emily R. - B60  
 Crowson-Hindman, Lauren R. - H76  
 Cruciotti, Juli A. - W07  
 Cruse, Courtney - B146  
 Cuenca, Daniela D. - W15  
 Cunha, Eugenia - A84

## D

Dahlberg, Alison F. - F1  
 Dalzell, Kourtney A. - B105  
 Dar, Mariam - D32  
 Darnell, James - W03  
 Darrisaw, Lora A. - H145  
 Dautartas, Angela M. - A42  
 Dautreme, Bérengère - K2  
 Davis, Kymeri E. - B77  
 De Aloe, Luigi - E61, E62, E63, E69, H46, H47, H68, H74, H88, I20  
 DeAngelis, Gina - C10  
 de Boer, Hans H. - H30  
 De Cree, Theresa M. - A16  
 De Donno, Marco - B116  
 DeGreeff, Lauryn - B19, E1  
 DeKalb Miller, Meredith - J22  
 De La Paz, Jade S. - A14  
 Del Fante, Zoe - H142

Dellaia, Domenico - G2  
 dell'Aquila, Massimiliano - H122  
 Della Rocca, Chiara - B63  
 DeMeter, Kaeleigh - H26  
 De Simone, Stefania - E49, E50, E51, E52, E57, F10, H59, H71  
 De Sousa, João - E43  
 Dempsey, Nick - A92  
 Dempsey, Sara - K33  
 Depp, Randi M. - E39  
 Derrick, Sharon M. - A82  
 DeRuyter, Emily A. - B97  
 Desiderio, Vincent J., Jr. - S02  
 Desranleau, Sylvain - G10  
 Dettman, Josh - W06  
 Deveci, Cemyigit - H108  
 Dickinson, Gregory M. - H40  
 DiEmma, Gabrielle E. - E21  
 DiGangi, Elizabeth A. - A1, A105, A110, Y6  
 Dinh, Nancy - B2, CB4  
 Dodt, Suzi - H95  
 Domanic, Matthew - W03  
 D'Orazio, Amanda - K27  
 Dougher, Meaghan C. - H138  
 Downing, Nancy R. - E23  
 Downs, J.C.U. - H41, W14  
 Drake, Stacy A. - E8, E87  
 Dunn, Rhian - A41  
 Dziejczak, Tomasz - J16

## E

Edwards, Amelia J. - A55  
 Eisenhart, Linda - J1, J31  
 Eldridge, Heidi - B116  
 Elliott, Simon - W06  
 Eng, Vanessa - B148  
 Epstein, Brandon - C15, W03  
 Esparza, Jessica M. - B120  
 Esposito, Massimiliano - H43, H87, H104  
 Evangelou, Elizabeth A. - A1  
 Evans, Alexandra - B21  
 Eze, Uwom O. - E84

## F

Falardeau, Mylène - B111, J35  
 Fallahi, Amin - C26  
 Farrell, Kaitlin - B86  
 Farris, Samuel J. - H64  
 Fauser, Rolf - J30



Feeser, Laney R. - A87  
Felthous, Alan R. - I17, I18  
Fenton, Emily - K25  
Feras, Khalid S. - B99  
Ferguson, Tracy - B57  
Fernandez Tejero, Nicole - B71  
Ferrara, Michela - E49, E50, E51, E52,  
E57, F10, H59, H71  
Ferrell, Morgan J. - E2  
Ferrero, Alessandro M. - D14  
Fielder, Angela G. - I19  
Fierro, Marcella F. - H36  
Finch, Eleanor - B160  
Fiorentin, Tais R. - B22  
Fischer, Manon - C6  
Fitzpatrick, Colleen M. - CB2, LW3, W05  
Flint, Casey A. - H15  
Fogarty, Melissa - W11  
Foley, Megan M. - B65  
Ford, Jonathan M. - H139  
Forman, Jonathan - W06  
Forry, Erin P. - W09  
Forte, Leann - B78  
Fortney, Amber - E74  
Fowble, Kristen L. - B145  
Fox, Bryanna - Y6  
Fox, Sherry C. - A85  
Franqueira, Virginia - W03  
Frauenhofer, Eric - A18  
Freeman, Adam J. - CB4  
Freitas, Corina - W18  
Friedman, Susan - CB4  
Fulginiti, Laura C. - A2  
Fulp, Kaitlyn J. - A74

## G

Gabaeff, Steven C. - H3  
Gaines, Allison - H83, I5  
Galimany, Jacqueline - A27  
Gallagher, Ryan - B58  
Gallegos, Shawna F. - B115  
Gallidabino, Matteo D. - B113  
Gandhi, Tanvi - C25  
Garcia, Lynn - F4  
Gardner, Brett O. - B24  
Garvin, Hannah - B153  
Garvin, Heather M. - A52  
Garza, Cynthia R. - F1  
Gault, Hunter N. - Y12  
Geiman, Irina - CB5  
Geissenberger, Janine - H115

George, Rebecca L. - A80  
Geradts, Zeno J. - W13, W14  
Gerber, Yana - J8  
Gettings, Katherine B. - W15  
Getz, Sara M. - A28  
Ghannam, Sarah H. - A24  
Gill, James R. - H27  
Gittelson, Simone - W10  
Gitto, Lorenzo - H9, H55, H79  
Giudice, Oliver - C2  
Gluodenis, Thomas J., Jr. - E77  
Glynn, Claire L. - B34  
Goff, Mark - J11  
Gogola, Nicholas - B87, B89  
Goliath, Jesse Roberto - A111  
Gonzalez, Mirtha L. - E36  
Goodson, Mark E. - H38  
Gorden, Erin M. - W15  
Gorniak, Jan M. - W14  
Gorski, Zuzanna - H31  
Gorski, Zygmunt M. - D18  
Gottfried, Emily D. - S02, W18  
Graham, Jason K. - SC1  
Gray, Teresa R. - K30  
Green, Richard - W15  
Greytak, Ellen M. - W15  
Grgicak, Catherine M. - B139  
Grigoras, Catalin - C11, W03, W13  
Grimsley, Kelly A. - F9  
Groshon, Nicole Marie - E6  
Grossweiler, Lisa L. - W15  
Guan, Yong - C13  
Guareschi, Edda - A94  
Guarnera, Luca - C2  
Guido, Mark D. - C24  
Guigne, Albane - E41  
Gulmen, Mete K. - E26, H62  
Gumpeni, Pramod - H26  
Gunther, Wendy M. - H57  
Gutierrez, Carlos A. - E27  
Guttman, Barbara - C8, W03  
Guynes, Keroshini - A5

## H

Ha, Helen H. - K48  
Haak, Lyndsay - C12  
Hacker, Alia S. - B28  
Hackett, Sarah B. - I16  
Haertel, Maryah E.M. - B158  
Hageman, Cecilia - F7  
Hagen, Dominik - H67

Hainsworth, Sarah V. - D23  
Hall, Mary L. - S02  
Hall, Teresa - CB4  
Haller, Leslie A. - G15  
Harding, Brett E. - E88  
Harkins Kincaid, Kelly - B35  
Harralson, Heidi H. - J21, J25  
Harran, Frederick - F17  
Harrington, Peter de B. - B159  
Harris, Annalivia - B118  
Hartley, Stephanie - A43, A44  
Hasert, Anita Roman - E89  
Hedges, Robert F. - F24  
Helm, Katharina - B62  
Hewitt, Curt - B7  
Hewitt, Terry-Dawn - F27  
Hicklin, R. Austin - J1  
Hicks, Tacha - W10  
Higgins, Brian - B41  
Higgins, Mairin M. - K22  
Hinnawi, Sarah - E32, E64, E82  
Ho, Jerika - H16  
Hofer, Peter - E20  
Hoke, Tiffany L. - Y11  
Höland, Katharina M. - K11  
Holoyda, Brian J. - W04  
Holt, Alaina - K24  
Holt, Cydne L. - W05  
Horsley, SaMoura L. - A36  
Horsman, Graeme - W03  
Horvath, Mary F. - W03  
Howe, James - W03  
Hu, John X. - H81, H95  
Huang, Ting-yu - B103  
Huard, Laurianne - B91  
Hudgins, Ashley - W08  
Huffman, Kaitlin S. - B135  
Hughes, Cris E. - A12  
Hulse, Cortney N. - A7  
Hundl, Callan - E80  
Hunter, Cheryl D. - S02  
Huston, Callie G. - H124

## I

Iancu, Lavinia - H13  
Iannaccone, Francesca - E47  
Iliescu, Anca R. - G9  
Innocenzi, Fabio - I13  
Ippoliti, Paul J. - D9  
Irion, Phillip - B73  
Isa, Mariyam I. - A91



## J

---

Jackson, David - B76  
Jackson, Linda C. - W17  
Jakob, Lisa - H118  
Jamshaid, Tahir - B84  
Jarman, Kristin - W06  
Jarvis, Hannah C. - H40  
Javan, Gulnaz T. - H128  
Jayaraman, Jayakumar - G18  
Jeanis, Michelle - Y6  
Jeerage, Kavita M. - D27  
Jelinek, Alexis - H69  
Jenkins, Amy Curtis - F3  
Jeong, Yangseung - A81  
Jimenez, Adam - H51  
Johnson, Keelie - J26  
Johnson, Madeline Q. - J20  
Johnson, Rachel A. - B119  
Jones, John P., II - SC1  
Joseph, A. Skylar - A100  
Joseph, Jacqueline A. - J18  
Juarez, Chelsey A. - A75

## K

---

Kacinko, Sherri L. - S01  
Kaempfen, Ricardo - H110  
Kalafut, Tim - F26, W02, W10  
Kalantzis, Niko - J12, J14, J15, J16, W01  
Kammrath, Brooke W. - B151  
Kanchan, Tanuj - A26, G22  
Kaplan, Molly A. - A17  
Kaszubinski, Sierra - H14  
Katsanis, Sara H. - A86  
Katz, Corey B. - B26  
Kavaloski, Heather - B50  
Kayser, Manfred - W15  
Kelley, Luz J. - B150  
Kellogg, Kevin C. - W05  
Kemper, Clarence B., III - D8  
Kennedy, Roderick T. - S01, W09  
Kenney Baden, Linda - W14  
Kerrigan, Sarah - B12, SC1, W17  
Keyes, Craig A. - A67, E15  
Keyes, Kelly - H29  
Kiely, Jennifer R. - A6  
Kim, Dong-Ho Eddie - A11  
King, Pamela A.W. - W09  
King, Steff - Y2  
Kirkosky, Scott E. - I18  
Klales, Alexandra R. - A15

Klein, Kaitlyn - A53  
Koester, Erin P. - E81  
Kortonick, Kristine A. - A59  
Krasniqi, Herolind - B98  
Krause, Michael - B162  
Krebs, Kyle - I5  
Kriiska, Kairi - J30  
Krishan, Kewal - E10, E12, E13  
Krotulski, Alex J. - K40, W11  
Kruijver, Maarten - B38  
Kuehn, Kelsi - A54  
Kwiatkowski, Sam - B37, H12

## L

---

La, Hae-Sun - H72  
Labay, Laura M. - K35, K53, S02  
L'Abbe, Ericka N. - A107  
LaFrance, Samantha - A47  
Lai, Yungchun - B44  
Lam, Vienna C. - E83  
Lamond, Victoria - A47, A59  
Lanfranchi, Anthony V. - A59  
LaPorte, Gerald M. - E76  
Laurent, François-Xavier - B123, W15  
Lawas, Maria Christiene M. - C32  
Layman, Andrew J. - H33  
Layne, Tiffany R. - B1  
LeBeau, Marc A. - W09  
Ledic, Andrea - J7  
Lee, Alan - B110  
Lee, Dayong - K32  
Lee, Jeff Cheng-Lung - F12  
Lee, Seungyeon - B4, B5  
Lemos, Nikolas P. - S02  
LeSassier, Danielle S. - B157  
Lewis, Allison - F13  
Lewis, Andra - B93  
Lewis, Paul Renfroe, Jr. - D12, D13  
Liebenberg, Maritza - A62  
Liliedahl, Ruby E. - B80  
Lim, Chin Chin - B144  
Lippincott, Lauren - H96  
Lloyd, John D. - D16  
Lockwood, Tracy-Lynn E. - B20  
Logan, Barry K. - W04, W11, W17  
Londino-Smolar, Gina - E73  
Long, Holly A. - A53  
Lopez, John F., Jr. - F1  
Lothridge, Kevin - E76  
Lucchi, John - E3  
Lusa, Vincenzo - I6

Lyle, James R. - W03  
Lynch, Robert Dale - D15  
Lyons, Bertram - C15, W03

## M

---

Machin, Ruth M. - H140  
Maclayton, Paulyann - H2  
MacMillan, Victoria - J34  
Mader, Jade - K18  
Madrzykowski, Daniel - D10  
Maggio, Ariane - A32  
Magidenko, Maria E. - H103  
Maglietta, Francesca - E49, E50, E51, E52, E54, E57, E58, F10, H59, H71  
Magno, Crystal - H82  
Maharramli, Shabnam - B67  
Mahoney, Pamela - I27  
Maidment, Catherine - A60  
Maiers, Justin R. - A13  
Makino, Yohsuke - H141  
Maloney, Katherine F. - H37  
Manetti, Alice Chiara - E46  
Manetti, Federico - H91, K21  
Manfredi, Alessandro - H109  
Manna, Modhuparna - C4  
Marco, Stephanie - K23  
Marks, David Brian - C19  
Marpet, Mark I. - D25, D26  
Marrone, Maricla - B100  
Marshall, Charla - W15  
Martin, Abby - C17  
Martin, Madison N. - K17  
Martinez, Carisa - B118  
Martinez, Diana - E35  
Mason, Allison R. - H114  
Mata, Dani C. - W11  
Mathew, Ashley E. - H6  
Mattei, Aldo - B23  
Mavroudas, Sophia - A31  
Maxwell, Ashley B. - A61  
May, Amanda - A96  
Mayfield, Amber L. - B56  
Mayo, Joshua - E81  
McClary, Carl R. - S02  
McEwen, Beverly J. - H136  
McGregor, Madison - Y10  
McKiel, Mary C. - W09  
McLamb, Jessica M. - B52, B53  
McLaren, Sterling - H7  
McMahon, Timothy P. - W15  
McPherson, Melinda K. - W08



# Presenting Author Index—2021

Medrano, Stephanie - A17, A37  
Mei, Nagi - C16  
Meiklejohn, Kelly A. - B130  
Mein, Caley A. - A71  
Meloro, Raphaela M. - A45  
Melson, Kenneth E. - W09  
Melton, Annamaria - H10  
Menard, Melissa - H101  
Menendez, M.J. - W04  
Merlino, Mara L. - J3  
Mesli, Vadim - A72  
Messer, Diana Lynn - A90  
Meydanci, Hacer Merve - E53  
Michael, Amy - A101  
Michon, Emily F. - A39  
Middleberg, Robert A. - K35, K53  
Midthun, Kari M. - K52  
Millette, James - D17  
Mills, DeEtta - E76  
Milroy, Chris - S01  
Miranker, Molly - A66  
Mirza, Mohammad M. - C27  
Mitchell, Randolph L. - G7  
Mitchell, Rebecca - B46  
Mitrani, Rebecca D. - B25  
Moiseff, Robin - H100  
Moldenhauer, Taylor R. - B10  
Mondello, Cristina - H61, H90, H123  
Montana, Angelo - H43  
Moore, Clarra G. - J19  
Moorthy, Arun S. - B17, B18, W07  
Moran, Kimberlee Sue - E75, W08  
Morel, Jessica - I5, W18  
Moretti, Matteo - K13  
Morioka, Fumiya - H125  
Morris, Richard - H53  
Moses, Sharon K. - E25  
Muir, Lurette - K8  
Mulder, Grace - H116  
Mullaney, Patricia - W03  
Mulloor, Jerome - B83

## N

Nead, Jacob D. - Y3  
Nestle, Emily - H129  
Nettles, Kirsten R. - B96  
Neuman, Maddisen - B117  
Neumark, Thomas - J2  
Nguyen, Lam D. - C19, C28  
Nicka, Catherine - H106  
Nieroda, Tarah - B133

Nirenberg, Michael S. - E11  
Nixon, John - D33  
Noble, Andrea Carolina - K47  
Novaes, Kathryn - E37  
Novak, Martin - F20  
Novroski, Nicole M.M. - B136  
Nugent, Kimberly - J34, J35  
Nuzzolese, Emilio - G2  
Nye, Natasha - B70

## O

O'Brien, Robert I. - B66  
O'Burke, James P. - E31  
Odom, Nicole R. - W03  
Oefelein, Rachel H. - F8  
Okura, Sonoka - D1  
Olckers, Antonel - B138  
Oldoni, Fabio - B42  
Olluri, Blerim - B98  
Olson, Larry A. - J28  
Oner, Dilara - J5  
Ordeman, Beth - SC1  
Osborne, Amy M. - B27  
Oulton, Scott R. - SC1

## P

Padgett, Brian D. - A69  
Padovano, Martina - H92  
Palmiotto, Andrea - A51  
Palmquist, Kaitlyn B. - K39  
Paolucci, John C. - E19  
Parchake, Manoj Bhausahab - H28, H94  
Parke, Leslie - E9  
Parker, Glendon - B124  
Parker, Joseph L., Sr. - J17  
Parr, Nicolette - A104  
Parson, Walther - W15  
Parsons, Hillary R. - A115  
Parsons, Thomas J. - W15  
Pascale, Natascha - E54, E58  
Pasham, Vishwajeeth - H111  
Passalacqua, Nicholas V. - A40  
Patel, Khusbu - H8  
Paulsen, Ryan B. - K16  
Pechal, Jennifer L. - H127  
Peck, Levi E. - Y12  
Perdue, Charla Skinner - W08  
Pereira de Barros, Magda A. - B3  
Perlin, Mark W. - S01  
Perner, Anja Victoria - H120

Perrillo, Richard J. - I27  
Pertsinakis, Michael - J15  
Peterson, Tynan - E40  
Pham, Amy - A88  
Phillips, Angelina I. - H75, H76, H111  
Pieczynski, Victoria - B61  
Pilloud, Marin A. - A103, A114, S02  
Pink, Christine M. - A83  
Pinto, Julie - A95  
Pinto, Sara Lo - H63  
Pipaliya, Royal - I1  
Pittner, Stefan - H119  
Pokorak, Eric G. - SC1  
Polston, Carrie - J29  
Pope, Elayne J. - A98  
Porterfield, Caitlin E. - E74  
Pozzi, Mark C. - D12, D13, D19, D21, D24  
Pozzi, Rachel L. - D19, D21, D24  
Preciado, Ringo - B104  
Pregent, Jenna M. - B92  
Pressley, DeMia P. - B16  
Prinz, Mechthild K. - W19  
Procopio, Noemi - B131  
Prusinowski, Meghan - B95

## Q

Quattrocchi, Alessia - H122  
Quenault, Aline - I25

## R

Radaelli, Davide - H70  
Raffaele, Roberto - E63, H47, Y7  
Rahm, Joseph M. - B74  
Ramos, Christopher - E85  
Ramsland, Katherine - E70  
Ray, Anyesha - J27  
Reber, Jami - K44  
Reczek, Karen - CB1  
Reedy, Paul - F23  
Reid, Sean M. - H52  
Remy, Joseph - W03  
Renegar, Thomas B. - B107  
Rentas, Cristina L. - B133  
Reyes-Rodriguez, Jenise - C8  
Rhinesmith-Carranza, Jennifer L. - H17  
Ricci, Pietrantonio - E17, E61, E62, E63, E69, H60, H68, H88, H93, H105, I15, I20, Y7  
Rich, Dayla C. - E4



# Presenting Author Index—2021

Rieders, Michael F. - K53, W04  
Righi, Fabiola - H32  
Rink, Stephanie - Y9  
Rippert, Andreas J. - J30  
Risemberg, Henry L. - C29  
Roberts, Katherine A. - F11  
Rocuzzo, Salvatore - H61, H123  
Rodda, Kabrena E. - W06  
Rodriguez-Cruz, Sandra E. - B13, SC1,  
W17  
Rogers, Caitlin E. - B125  
Rogers, Marcus - C7  
Rolf, Cristin Marie - H11  
Roper-Miller, Jeri D. - S02, W06  
Ross, Ann H. - A114  
Ross, Jarrett P. - B36  
Rowbotham, Samantha K. - A89  
Rowe, Walter F. - B156, J32  
Roy, Casey - E68  
Rudin, Leonid I. - W13  
Rueckert, Justin - H107  
Ruengdit, Sittiporn - A29  
Runge, Teresa M. - A30  
Russ, Michael - E22  
Russell, David - B72  
Rutten, Lenora N. - J33  
Rydzak, Patrick M. - B147

## S

Saayman, Gert - H39  
Sacco, Matteo A. - E61, E62, E66, E67,  
H23, H46, H60, H68, H74, H88, H93,  
I20, Y7  
Saczalski, Kenneth J. - D13, D21  
Sakelliadis, Emmanouil I. - H135  
Salerno, Monica - H85, H126  
Sanford, Michelle R. - E7  
Sanger, Robert M. - F21, S02  
Santillan, Perla - A58  
Santoro, Paola - H112  
Santos Martins, Celeste - H1  
Santurro, Alessandro - H49  
Sawyer, Samantha J. - H18  
Scafide, Katherine N. - E23  
Scalise, Carmen - E61, E62, E63, E66,  
E67, E69, H47, H68, H74, H88, H105,  
I15, Y7  
Scatena, Andrea - E47  
Scendonì, Roberto - A22  
Schaeffer, Luther S. - W07  
Scheible, Melissa K.R. - B54

Schellhammer, Sarah K. - B32  
Scherr, Albert (Buzz) E. - F15  
Schjonsby, Hakon - J30  
Schmid, John D. - F14  
Schmidt, Carl J. - W04  
Schrade, Brad - W14  
Schroeder, William M., II - K9  
Schwalbach, Nicole H.W. - A64  
Sciarretta, Marybeth J. - B36  
Scott, Kirstie R. - B132  
Scotti, Veronica - F18  
Seaman Kelly, Jan - J10  
Sebetan, Ismail M. - E35, E37, F9, H124  
Seidel, Andrew C. - A2  
Seki, Yoko - J23  
Sellnau, Natalie - B85  
Semaan, Marie - B137  
Serinelli, Serenella - H9, H55, H79  
Sessa, Francesco - E49, H85, H126  
Sgan, Stephen L. - H24  
Sgheiza, Valerie - A77  
Shahid, Mehdi - C23  
Sharp, Nicholas - D6  
Shedge, Rutwik D. - A21, A23  
Shelton, Donald E. - F6  
Sheth, Nidhi - B49  
Shi, Chen - C13  
Shiple, Summer B. - A53  
Shnaidman, Vivian - W18  
Shonsey, Erin - W07  
Shu, Yea Lee - G11  
Shute, Rebecca - E22  
Sicilia, Francesco - E61, E62, H46, H60,  
H68, H74, H88, H89, H93, H105, I15,  
I20  
Sigman, Michael E. - B141  
Simmons, Hannah Noël - S02  
Simmons-Ehrhardt, Terrie - A49  
Simon, Botond - G13  
Sims, Lora - C20  
Singer, Andrew - F16  
Sinha, Sudhir K. - B127  
Siodambro, Chiara - K13  
Sisco, Edward - W07  
Skillman, Britni N. - K3  
Skipper, Cassie E. - A36  
Skokan, Lilian - B114  
Slayton, David - F28  
Smith, Christina - K42  
Smith, Erich D. - B108  
Smith, Keenen - H76  
Smith, Kendall - H98

Smith, Patricia C. - CB3  
Sofalvi, Szabolcs - W11  
Somogyi, Tessa - A1  
Soto, Fatima - A68  
Soto Martinez, Miriam E. - A3  
Souviron, Richard R. - G15  
Sowemimo, Oluwakemi - B39  
Spamer, Bobbie B.J. - E29  
Speaker, Paul - E79, K4  
Spear, Brianna - K26  
Speth, Pete - LW4  
Spies, Amy Joy - A93  
Spiros, Micayla C. - A99  
Springer, Kelsey - H117  
Srettabunjong, Supawon - B68  
Stacy, Megan - I5  
Starck, Abigail J. - B154  
Stassi, Chiara - H130, Y5  
Staton, Jerry R. - W14  
Stein, Paul - E35, E37, F9, H124  
Stellacci, Alessandra - B100  
Stephens, Joseph C. - J31  
Stern, Hal S. - D2  
Stern, Libby A. - B149, B152  
Stock, Michala K. - A79  
Stolorow, Mark D. - F4  
Stone, Anne - H131  
Stowe, Neil - K20  
Stram, Michelle - H132  
Strickland, Erin C. - K6  
Strömmer, Ellen M.F. - H133  
Stubblefield, Phoebe R. - A112  
Sudhakaran, Sneha - C14  
Suiter, Christopher L. - B79  
Sun, Mingjing - K45  
Swartz, Marvin S. - I18  
Swift, Katie - E14  
Swortwood, Madeleine J. - W11

## T

Taglia, Fabiana - B48  
Tahirukaj, Milazim - B98  
Tanaka, Tobin A. - J34, J35, S02  
Tanzi, Raimo - B45  
Tarantino, Francesca - B100  
Tasso-Thompson, Rikki A. - Y12  
Tattoli, Lucia - I9, I10, I14  
Taylor, Lois S. - H20  
Terpstra, Toby M. - C21, C22  
Tettamanti, Camilla - E42  
Thoene, Caitlyn - E44



# Presenting Author Index—2021

Thomas, Santana A.L. - B33  
Thomas, Sarah C. - H73  
Thompson, Tim - W08  
Tiffon Nonis, Bernat-Noël - I21, I22, I23, I24  
Tillmar, Andreas - W15  
Tiscione, Nicholas B. - K31  
Tomlin, Leigh M. - F2  
Torsello, Alessandra - E59, E60, H71  
Tran, Cynthia J. Kaeser - W08  
Trask, Willa R. - A8  
Traustason, Andri - H44  
Troccoli, Giuseppe - I5  
Truver, Michael - K41  
Tsiatis, Nikolaos - H135  
Tumram, Nilesh Keshav - H66  
Turco, Sara - E86  
Turri Swatek, Jennifer L. - K50, K53  
Tyagi, Shashank - H86  
Tyner, Tamara - A57  
Tzvetanov, Krassimir - C9

## U

Ünsal, Tugba - B69, B75  
Urbas, Aaron - B15

## V

Valdez, Dana M.C. - I3  
Valentinuz, Eleonora - E16  
Valerio, Raymond - F30  
Vander Pyl, Courtney H. - B102  
Vareed, Shaiju - K5  
Vastrick, Dirk - S02  
Vastrick, Thomas W. - J6  
Velez, Aracelis A. - K38  
Veneri, Federico - B90  
Ventura Spagnolo, Elvira - H123  
Vidaki, Athina - W15  
Vieira, Duarte Nuno - H48, S02

Viel, Edith - K28, K43  
Vikingsson, Svante - K36, W11  
Vinci, Francesco - B100  
Vir, Muskan - J35  
Volonnino, Gianpietro - K15  
Volpini, Laura - I2  
Volz, Jessica - E14  
Vorburger, Theodore V. - B155

## W

Walcott, Robert C. - G12  
Wales, Gregory S. - W13  
Wall, Elora C.D. - B106  
Wallin, Jeanette M. - W05  
Walsh, Susan - W15  
Walter, Brittany S. - A24  
Wang, John Z. - E5  
Wang, Ling - B48  
Ward, Tabitha - H35  
Warriner, Varsha - A25  
Watkins, Rachel - A116  
Watson, Steven B. - W03  
Waxenbaum, Erin B. - A35  
Weedn, Victor W. - S01, W04  
Weiss, Kurt D. - D20  
Welker, Katrina - B56  
Wells, Kyla J. - F29  
Wendt, Frank R. - B136  
Wenzlow, Nanny - H65, H137  
Weyh, McKenzie - J9  
Weyrauch, Deland - H134  
Whitaker, Jonathan - W10  
White, Thomas R. - B112  
Whitecotton, Cole - C1, W03, W13  
Whitmer, Sean D. - B25  
Wiersema, Jason M. - W09  
Willi, Joseph - D11  
Williams, Amanda N. - A97  
Williams, Mary R. - B140  
Willis, Sheila - W10

Wilson, Lauren - E28  
Wilson, Sophena S. - C18  
Wilson, Teresa V. - A113  
Wilson, Walter Brent - D3, D28, W17  
Wilson-Taylor, Rebecca J. - A8  
Winecker, Ruth E. - K46  
Winokur, Agnes D. - S01, W04, W17  
Wise, Natalie M. - Y14  
Wood, Michelle - B82  
Wood, Robert E. - G6  
Woollen, Katharine C. - A73  
Word, Charlotte J. - W19  
Worrell, Erin M. - W04  
Wright, Diana M. - B29

## Y

Yalçin Saribey, Aylin - E38  
Yarberry, Andrea - B83  
Yeatman, Dustin Tate - SC1  
Yim, An-Di - A77  
Yount, Paul M. - G3  
Yukyi, Nandar - A102  
Yung, Jeffrey - H21

## Z

Zaferes, Andrea - H144  
Zambrano, Carlos J. - A65  
Zaney, Mary E. - W04  
Zangari, Francesca - G5  
Zanon, Martina - H80  
Zeigler, Karen - H58  
Zhang, Mengliang - B88  
Zhang, Xin - H99  
Zibetti, Angelica - E61, E62, E67, E69, H60, H93, H105, Y7  
Ziogos, Sotirios - H113  
Zlotnick, Joel A. - W16  
Zou, Tong - D2