# ℘ROCEEDINGS

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# *PROCEEDINGS*

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## Contents

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# H

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## K

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   ZyGEM Corporation, Ltd. (Discussion of Commercial Products
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C anine STR Reagent Kit (Discussion of Unlabeled/Investigational
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   Hitachi Software Engineering Co., Ltd. (Paid Consultant)
   New Skeletal Remains DNA Extraction Kit (Discussion of
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## O

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## S1  "NamUs"- The National Missing and Unidentified Persons System:  Resolving our Nation's Silent Mass Disaster

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In 2004, a national Bureau of Justices Statistics survey found that of the 4,400 unidentified human bodies received in medical examiner and coroner offices in an average year, 1,000 remain unidentified after one year.  It is an ongoing national problem that has historically been perpetuated by an inability to maximize resources and to share information among those charged with identifying remains and those conducting the investigations.

Many of the estimated 40,000 unidentified dead are homicide victims.  Detectives are increasingly overwhelmed with growing backlogs of cold cases involving nameless victims, hundreds of perpetrators are literally "getting away with murder," and somewhere, thousands of people are looking for their loved one(s).

In 2006, the National Center for Forensic Science, funded by a NIJ initiative, assembled a focus group of medical examiners, coroners, death investigators and nationally recognized forensic and technology experts to assess the unidentified persons problem and make recommendations.  The plea for standard practices and a free, accessible, searchable, online Unidentified Decedent Database was heard.

In July 2007, NIJ launched the National Missing and Unidentified Persons System (NamUs).  Medical Examiners, coroners, or their designees have to date entered 2,072 cases, of which 31 have been identified. The NamUs – Missing Persons Database is in development.

Upon attending this session, participants will understand why it is important that all agencies follow best practice standards to ensure that proper and necessary steps are taken to aid in identification.  Sequence of the steps, necessary procedures, and agency cooperation will be discussed.  Helpful checklists, forms and other resources will be made available.

The NamUs–Unidentified database will be thoroughly demonstrated and participants will have hands-on training in case data entry. Participants should bring laptop computers to use for this section of the special session.

**Human Identification, Unidentified Decedent database, ational Missing and Unidentified Persons System**

## S3  Young Forensic Scientists Forum - AAFS at a Glance:  Experience the Forensic Sciences

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Throughout the past eleven years the Young Forensic Scientists Forum has provided a program for a group of Academy members ranging from students to professionals who are new to their careers in forensic science. The program has grown and changed drastically in order to provide students and scientists who have five years experience or less with the highest quality information possible.  The continuing goal of this program is to provide this audience with topics relevant to their education, training, and skill levels.  The event also provides a comfortable means for students and professionals new to their respective fields a venue in which they may communicate with experienced Members and Fellows of the AAFS.  The session planned for the 61st Annual Scientific Meeting in Denver, Colorado, focuses on the broad range of forensic disciplines that the AAFS represents — with the theme "AAFS at a Glance:  Experience the Forensic Sciences."  Speakers from each forensic discipline will share their experience, casework, and research in order to give participants a thorough representation of the forensic science community. Following the day-long session, the program will continue with an evening session titled "Young Forensic Scientists Forum Poster Session."  The poster session will feature

posters by undergraduate and graduate students as well as forensic science professionals. The poster session will also present new, emerging forensic research and technologies to attendees. The event will allow young and emerging scientists to mingle with peers as well as established members of the AAFS in a comfortable setting.

The annual YFSF Bring Your Own Slides Session, with presentations from students and emerging forensic scientists, is scheduled for Wednesday evening. The program will continue Thursday morning with the annual YFSF Breakfast Meeting with a CV/resume review and various job related presentations. The presenters will focus on a variety of topics relating to the importance of professionalism when emerging into the forensic science field and will share their knowledge with participants through an open question and answer forum discussion.

It is the goal of the YFSF to foster relationships between the participants of the session with peers as well as established members of AAFS and to provide for a smooth transition from student, to emerging scientist, to established member. With the forum group setting provided and the variety of programs offered throughout the week, the YFSF will not only provide academic and relevant technical information to attendees, but will also cultivate relationships that will last a career.

**YFSF, Special, Session**

## ES1    New Investigative Techniques and Scientific Advancements for Forensic Scientists in the Future

*Cyril H. Wecht, MD, JD\*, 1119 Penn Avenue, #404, Pittsburgh, PA 15222-4205; Henry C. Lee, PhD\*, Forensic Laboratory, 278 Colony Street, Meriden, CT 06451; and Michael M. Baden, MD\*, 15 West 53rd Street, #18B-C, New York, NY 10019*

With the utilization of new techniques and equipment for more precise analyses and scientific resolution of controversial, contested, criminal, and civil litigation, forensic scientists as a result will be enabled to make greater contributions in the worlds of academia, medicine, law, and justice.

Scientific advancements are now evolving at an exponential pace. The seemingly fantastic predictions of Leonardo DaVinci and Jules Verne took centuries before becoming realities. Furthermore, the dramatically exciting extraterrestrial adventures of Flash Gordon and Buck Rogers that many of us enjoyed as kids required several decades to come to fruition. In the near future, there will truly be a new world of forensic science. However, some basic aspects of human society, both man-made and natural disasters, quite regrettably, are not likely to change. Disease and death, and crime and murder, will always be present. Unless our civilization is unpredictably altered to an extreme degree, there will be a continuing need for physicians, forensic scientists, and attorneys to cope with all of the medical and legal questions and controversies emanating from many of these natural and human tragedies that will become matters to be resolved within the criminal and civil justice systems.

To what extent can we realistically envision and thereby prepare for and constructively contribute to the creation of these future forensic endeavors? What can we learn in retrospect when we revisit some of the more infamous, controversial, and intellectually frustrating cases of the past, and contemplate how we might deal with them if they were to occur in the years ahead when many of the anticipated technological developments become procedural realities?

> How many shots were fired at JFK; can the "single bullet" theory be scientifically and unequivocally corroborated or disproven?
>
> Where exactly was Sirhan Sirhan standing when he fired his gun at RFK; and, was there a second shooter?

> What exactly happened in Phil Spector's home? Was the death of Lana Clarkson a suicide, accident, or homicide?
>
> What happened that resulted in the death of JonBenet Ramsey?
>
> Exactly where, when, and how was Laci Peterson killed?
>
> What were the precise quantities of the various drugs found in the dead patients at Memorial Hospital in the aftermath of Hurricane Katrina; and, exactly how and when were the drugs administered by Dr. Pou?
>
> Will the Innocence Project close shop by 2020 because DNA testing has freed every innocent person in jail? Is it possible to have an international universal database for every category of physical evidence?
>
> Will unearthed dental and skeletal remains and decomposing bodies be analyzed by experts utilizing new techniques with such precision as to enable facile determinations to be made regarding when, where, and how those individuals died?
>
> Will research studies of genetic profiles, human patterns of behavior, and the intricate workings of the brain have advanced to such an extent that they can be utilized to analyze deceptions of truth, analyze criminal acts, scientifically predict, and possibly even specifically identify the perpetrator?

Computer technology, iPod, image enhancement, artificial intelligence, and data mining are just a few of the interesting and provocative questions that all of us as forensic scientists should think about as our society continues to move forward at an ever increasing pace in the realm of investigative forensic science. Is electronic technology going to change forensic laboratory procedures and the complexion of crimes?

The presenters will discuss many of the prominent cases in which they have been involved in past years that continue to be subjects of much interest and controversy at the present time. These will be reviewed through the prism of future technology, as the presenters reflect upon what could have been learned when these cases occurred, and what still may be determined that could be relevant in ultimately resolving these cases in the future.

**Forensic, Technology, Future Application**

## ES2    ASCLD/LAB Symposium - Principles of Professional Responsibility for Crime Laboratories and Forensic Scientists

*Joseph P. Bono, MA\*, Science Program, IUPUI, 402 North Blackford Street, Indianapolis, IN 46202; Kenneth E. Melson, JD\*, Executive Office of the U.S. Attorney, 950 Pennsylvania Avenue, NW, #2261, Washington, DC 20530; Jay A. Siegel, PhD\*, IU, Purdue University Indianapolis, Chemistry, School of Science, 402 North Blackford, LD 326 D, Indianapolis, IN 46202; and Peter Neufeld, JD\*, Cochran, Neufeld & Scheck, LLP, 99 Hudson Street, 8th Floor, New York, NY 10013*

This symposium is a continuation of a program initiated approximately five years ago to discuss issues relevant to all forensic science laboratories. While this program is especially pertinent to laboratories accredited by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB), all practicing forensic scientists will benefit by attending this symposium and joining in the ensuing discussion.

ASCLD/LAB has adopted a document entitled ASCLD/LAB Guiding Principles of Professional Responsibility for Crime Laboratories and Forensic Scientists. This document addresses ethical and professional responsibilities in the forensic laboratory community. While not all inclusive, these "principles" describe key areas and provide some specific

rules to supplement existing codes of ethics adopted by recognized professional organizations and individual laboratories. The "Guiding Principles" are designed to enhance public confidence in the quality of forensic laboratory services, whether or not the laboratory is accredited by any accrediting body. The draft guidelines are posted at www.ascld-lab.org. Reviewing the guidelines before attending the session will help in facilitating a meaningful discussion.

The public and judicial confidence in a laboratory's work product is based on the credibility of the examiners who provide the forensic services. Without this confidence, the effects of good science are marginalized. The "Guiding Principles" are designed to enhance confidence in the quality of forensic laboratory services. Furthermore, developing and implementing a generally accepted code of professional responsibility will assist in supporting forensic scientists in exercising their professional responsibilities and encouraging laboratory management to create a culture of ethical and professional excellence in forensic laboratories.

ASCLD/LAB has identified three major areas relevant to a forensic scientist's practice: professionalism, competency and proficiency, and clear communications. In each of these areas, specific guidelines have been developed.

Ethical behavior is difficult to define; however, unethical behavior is recognized when it occurs. Does compliance with a legal requirement guarantee ethical behavior? Is the obligation of the forensic scientist in the courtroom different than the obligation of the lawyer (prosecutor, defense, and judge)? Does the right to express a valid opinion outweigh the obligation to express an opinion which considers all sides of an issue?

In many professions, ethical issues are true dilemmas. The right of an individual to practice can and does conflict with boundaries of acceptable behavior. In medicine, law, industry and academia, discussions related to ethics have become the norm, and in some instances, requirements. A discussion of ethics in forensic science laboratories should also be a requirement. Too much is at stake to condone unethical behavior by passive acceptance.

These and other issues will be discussed among a panel of distinguished experts. There will also be an opportunity for comment from those who attend the symposium.

**Ethics, ASCLD/LAB, Professional Responsibility**

## BS1 Stories Behind the Evolving Story on Heparin Related Deaths: Just Opportunism or Unrestricted Capitalism

*Abraham T. Philip, MD\*, Onondaga County Medical Examiner's Office, 100 Elizabeth Blackwell Street, Syracuse, NY 13210; and Jeanna M. Marraffa, PharmD, Upstate New York Poison Center, 250 Harrison Street, Syracuse, NY 13210*

After attending this presentation, attendees will gain an understanding of the causes, circumstances, and other issues involving the recent spate of heparin related deaths that have been in the news, as well as the topic of Congressional hearings.

This presentation will impact the forensic science community by providing details of the issues in the production of heparin. These issues have been the cause of adverse reactions to heparin therapy in over 800 people worldwide and at least 80 deaths. This presentation will explore the causes of failures in the process of obtaining raw materials for the manufacture of pharmaceuticals in the Western world.

The discovery of heparin, like so many other biologically active substances, was made serendipitously in the early years of the 19th century. A major worldwide research effort was involved in the development of this anticoagulant medication. Today, the drug is used in many medical conditions and in almost all surgeries lasting over 30 minutes.

Heparin, though initially manufactured in North America and Europe, is currently manufactured in developing countries due to lower costs. Procedures for extraction of heparin also changed from the liver of bovine lungs to porcine intestines. Currently, China dominates the heparin production market, supplying more than half the world's demand. The ever increasing global demand, an epidemic within the swine population, and other market forces converted large suppliers into consolidators while actual production was done in small unregulated food manufacturing units.

There is extensive processing of heparin extract in its journey from abattoir to the IV bag. Contamination of heparin with "over sulfated chondroitin sulfate" at the beginning of its supply chain led to allergic reactions and subsequent deaths became evident in the latter half of 2007. In a set of elegant experiments by Kishimoto et al., a link was established between the contaminant and the adverse reactions. The FDA developed new techniques for identifying the contaminants and the guidelines for the manufacturing and testing have been changed.

There are several unresolved issues about the contamination of heparin. Was it just accidental? Sabotage? Sheer opportunism? These questions and other controversial and provocative issues relating to this issue should be of significant relevance to members of the forensic and medico-legal communities because of the profound effects it has on so many patients worldwide.

**Heparin Related Deaths, Oversulfated Chondroitin Sulfate, Contamination**

## BS2 The Real Mason Verger: The Man Who Fed His Face to the Dogs

*Vernon J. Geberth, MS, MPS\*, PHI Investigative Consultants, Inc., PO Box 197, Garnerville, NY 10923*

After attending this presentation, attendees will appreciate the impact of an overdose of hallucinatory drugs. The goal of this presentation is to present to the members of the forensic community a bizarre and unique case of self-mutilation involving a man under the influence of PCP. The man literally sliced off his entire face with pieces of broken mirror and fed the flesh to the dogs. The man survived due to the analgesic properties of the drug phencyclidine, which can cause users to feel less pain, as well as the excellent medical care he received which included facial reconstruction using a pectoral flap procedure.

This presentation will impact the forensic community by examining this highly unusual case and demonstrating the need to conduct a complete and thorough investigation to ascertain the entirety of the circumstances surrounding such a bizarre event. The principle of taking each point to its logical conclusion proved invaluable when unexpectedly this self-mutilation became a theme in an award winning Hollywood movie entitled, "Hannibal," as well as a topic of discussion on the internet. Mason Verger was a fictional character in the novel "Hannibal" written by Thomas Harris about a despicable character and avowed pedophile who was hideously disfigured by the serial killer Dr. Hannibal Lector. In the novel while Verger was demonstrating his autoerotic asphyxiation, Dr. Lector offered him amyl nitrate and several other mind-altering drugs convincing Verger to tear his face off with a shard of mirror and feed it to his pet dogs. Verger does so, also gouging out one of his eyes, and eating his own nose. So, "Does Art Imitate Life? Or, Does Life Imitate Art"? This scenario was literally taken right out of the author's textbook by Mr. Harris without acknowledgement or citation of source and is actually based on a case that the author investigated as a commander in the NYPD.

PCP has potent effects on the nervous system, altering perceptual functions (hallucinations, delusional ideas, delirium, and/or confused thinking). The drug has been known to alter mood states in an unpredictable fashion, causing some individuals to become detached and others to become animated. Intoxicated individuals may act in an unpredictable fashion, driven by their delusions or hallucinations. Included in the portfolio of behavioral disturbances are acts of self-injury including suicide, and attacks on others or destruction of property.

This bizarre self-mutilation case concerned a young man named Michael who was high on "Angel Dust" or phencyclidine (PCP). PCP is a powerful psychedelic and anesthetic drug known for its dissociative effects at higher doses. It is also associated with the strange and sometimes violent behaviors of people under its influence.

While under the influence of the PCP, Michael had taken his clothes off at a woman's apartment. He began to act strange and was "talking nonsense." PCP gives a feeling of being disconnected from one's body and environment. After his actions with the woman in the apartment, another male neighbor asked him to leave and directed him back to his basement apartment. Michael apparently continued to use the PCP, which obviously induced a psychotic state. There was evidence that he had smashed a mirror, which he then used to mutilate himself. The analgesic properties of the drug can cause users to feel less pain and persist in violent or injurious acts. The investigation revealed that Michael had literally fed his face to the dogs that were in the basement

and gouged out one of his eyes with a piece of glass from the mirror that he had smashed.

**The Investigation:** The investigation began with simultaneous calls from North Central Bronx Hospital and the patrol sergeant at the crime scene both requesting detectives. EMS had transported the seriously injured and mutilated man under the influence of drugs to the hospital. Michael told EMS, "The dogs did it," then passed out. At the hospital as he was being stabilized, he mumbled something about movies and that, "Someone was trying to peel off his face." He then uttered, "I did it myself. It's an offering to Big Bird."

The crime scene had been secured by the patrol sergeant, who reported that they had also locked some dogs in a back room of the basement. EMS had removed the victim from the bathroom after uniform officers had corralled the dogs. Detectives noted that there was little blood in the bathroom considering the extensive injuries of the victim. At this point investigators were still considering this case a possible assault due to these extensive injuries.

Detectives were able to determine that the actual cutting took place in the living room area, specifically on a reclining leather chair. There was blood soaked into the chair and pieces of a smashed mirror on the floor with blood drops as well as bloody fingerprints. One shard of mirror glass had been used by the victim to peel his face and had a partial print on it. These fingerprints were matched to the victim's prints, which were on file from previous drug arrests. Examination of the crime scene revealed that after the victim had peeled his face, he had apparently laid down on the basement floor with the three dogs. Detectives located the dog's owner who gave them permission to take the dogs to the ASPCA for forensic examination. The ASPCA was contacted and were requested to have a doctor available to examine an adult female german shepherd and two puppies. The veterinarian induced vomiting, which resulted in the recovery of human tissue consisting of pieces of the victim's lips, skin, and nose.

**Investigation at the Hospital:** As the investigation continued at the hospital, the emergency room was filled with doctors and nurses attending to the mutilated victim. The man's face had been wrapped with moistened gauze strips and the medical personnel were administering an IV as he was being monitored.

Detectives photographed the victim and his injuries and informed the Emergency Room doctors of what had transpired at the scene as well as the medical operations at ASPCA. Detectives noticed that the victim had gouged out one eye and the other eyeball was sitting on his face like a cyclops. The detectives had brought the pieces of flesh that the ASPCA doctor had removed from the dog's stomachs to the emergency room. However, these materials were in no condition for grafting. Detectives advised the doctors that they would try to get a statement from the subject in their presence. The doctors were asked to remove the gauze from the victim's mouth so that the subject could attempt to talk. As one of the detectives spoke into the subject's ear, "What happened to you"? The subject suddenly began to mumble and then shout, "AYAH, AYAH, AYAH, AYAH" over and over again with his teeth opening and closing like a mechanical box.

**The Medical Aspects:** The man survived even though he had peeled his face from his skull. Apparently, the PCP had provided the victim with an anesthetizing effect during his self-mutilation. However, the amount of drugs he had ingested had also damaged his brain function. He became a "Ward of the State." He also became the plastic surgeon's major project as they began to implement reconstructive surgery using a pectoral flap procedure. During the procedure, the pectoral (chest) muscle is removed and implanted at another site on the victim's body. The surgeons leave the artery and veins intact. The muscle is then "flapped" to the site and sewn into place where it eventually grows a new blood supply. Michael had two pectoral flaps done one from each side of his chest to each side of his face. Once the muscles were established in their new location the surgeons would cut and revise the grafts to create a new face for the subject.

**Apparently Art Does Imitate Life:** When Thomas Harris' book "Hannibal" and the subsequent movie came out, a number of news outlets made inquiries about the development of this character. Mason Verger was obviously based on "Michael" and the character and story obviously been taken from Practical Homicide Investigation: Tactics, Procedures and Forensic Techniques (CRC Press, LLC). The author contacted Mr. Harris who did acknowledge that Mason Verger was in fact based on the case in Practical Homicide Investigation and is now cited in the subsequent printings.

**Conclusion:** This presentation and discussion should clear up any misconceptions or errors that some outside self-appointed experts have rendered. This investigation, which is a matter of official record, was completely documented and each point was taken to its logical conclusion. This presentation affords the forensic community with a frame of reference for a unique and bizarre case of self-mutilation of a man who was able to inflict such devastating injuries and survive due to the analgesic properties of an overdose of phencyclidine.

**Drug Induced Self Mutilation, PCP, Pectoral Flap Procedure**

## BS3 The Seven Deadly Sins of Forensic Practice

*J.C. Upshaw Downs, MD\*, Georgia Bureau of Investigation CRCL, 925A Mohawk Street, Savannah, GA 31419*

After attending this presentation, attendees will consider several broad areas (within the field of medicolegal death investigation) of potential ethical and/or performance concerns and possible means by which to prevent such pitfalls.

This presentation will impact the forensic science community by exposing practitioners to a paradigm of potential pitfalls and means by which to avoid them in order to improve the quality of case work.

A forensic science career is wrought with potential dangers for the practitioner. The quest to apply pure science in a legal setting, as a noble goal, can follow a treacherous course. Not least among the snares are questions of applied ethics. Although a broad subject, well beyond the scope of a brief overview, one may stratify shortcomings along many lines, including "minor" or "major" transgressions. In ancient times, some came to view these poles as represented by the "venial" or relatively inconsequential and the "capital" or serious offenses. The latter came to be known as the seven deadly sins.

Although not specifically mentioned in biblical references, brief mention can be found in the book of Proverbs (6:16-19) of a basis for the codification of offenses. The Roman Catholic Church espoused a virtuous life and avoidance of all evils, particularly the capital offenses. Through the years, the precise meaning of the various terms has evolved, although the general concepts have remained intact. The seven deadly sins as described by Dante in The Divine Comedy include luxuria (extravagance or lust), gula (gluttony), avaritia (greed), acedia (sloth), ira (wrath or anger), invidia (envy), and superbia (pride). The appeal of the darker side of human nature is evident in the popularization of these traits in the form of popular movies and television series.

Classically, each sin has a contrasting cardinal virtue: humility, kindness, abstinence, chastity, patience, generosity, and diligence. Examples where the opposing virtue might better serve the case at hand will seek to help practitioners remain focused on the ideals of forensic practice.

Avoiding a religious treatise, working modern definitions of the sins are presented. Utilizing a case-based approach, each of these hazards will be discussed with emphasis on the patterns of behavior possibly ending in the undesirable outcome for the case and the justice system. As "those who fail to learn from the mistakes of the past are doomed to repeat them," this series of cautionary tales is intended to challenge the attendee by serving as a reminder of the wisdom of the ages in considering one's ethical

foundation and views on casework, hopefully thereby strengthening individual practitioner's resolve to better serve the populace.

**Ethics, Pitfalls, Medicolegal**

## BS4  Breakfast With Alferd G. Packer:  Colorado's Premier Cannibal

*Jane H. Bock, PhD*, EE Biology Department, University of Colorado, Box 334, Boulder, CO 80309-0334; and David O. Norris, PhD*, Department of Integrative Physiology, Campus Box 354, University of Colorado, Boulder, CO 80309-0354*

After attending this presentation, attendees will be familiar with one of the more unusual true stories in Colorado's not so distant past.

This presentation will impact the forensic science community by showing how Colorado's legal system dealt with exceedingly difficult matters that at the time lacked useful precedents.

Alferd G. Packer was born in Pennsylvania in 1842 and died in 1907, and is buried in Littleton, Colorado.  In 1873, he came to Colorado during the gold rush looking for wealth.  In early 1874, he and five companions left for Gunnison, Colorado, against the advice of locals because of the dangerous winter weather in the Rockies. They got lost and were snowbound a few weeks later.  Packer reappeared alone in April of that year and claimed that one of his companions had gone mad and eaten the other companions while Packer was away looking for a trail out of the mountains.  In August of 1874, Packer signed a confession, escaped from jail, and in 1883 was found in Wyoming living under an alias.  He was brought back to Colorado for trial and was found guilty.  The Colorado Supreme Court reversed the conviction and in a retrial in 1899 he was sentenced to a 40 year prison term.  Packer was paroled in 1905 and two years later died of natural causes.  One of his monuments is at the University of Colorado's Boulder campus where the Alferd G. Packer Grill continues to serve thousands of meals.

**Cannibal, Prospectors, Serial Killers**

## BS5  Departure Angle From Roadway Using Vault Calculations

*Robert L. Anderson, BSME*, Applied Research and Investigations, PO Box 1208, Scottsdale, AZ 85252*

After attending this presentation, attendees will understand an unconventional method for determining departure angle from roadway when the roadside has a steep cross slope.

This paper will impact the forensic science community by providing a method for calculating departure angle with limited information.

Roadway design cases sometimes involve an issue of whether a guard rail should be required.  Frequently these cases require the reconstruction of the departure angle.  The angle of contact with the guard rail determines the effectiveness of the guard rail.  The tire marks on the roadway may not be documented.  If the roadside has a steep drop off, the vehicle will vault off of the roadway and then leave tire marks off of the roadway.  The steep terrain makes the accurate documentation of these marks by police officers difficult.  This is particularly true if the police do the measurements manually.

A case study is presented that illustrates the use of the vault formulas to calculate a departure angle.  This case involved a vehicle that went off of the right hand side of the road without leaving marks that were documented by either police photographs or measurements. The police measurements of tire marks off of the roadway were clearly in error.  A survey of the scene after the accident and after the physical

evidence was missing, showed that the tire marks as documented by the police, being partially in space over a drop off.

A contour map was generated from the post-accident survey and the beginning of the off road tire marks documented.  It was assumed that the start of the marks were reasonably accurate since they were closest to the road both laterally and vertically. The change in elevation from the road surface to the start of the tire marks was measured from the contour map.

This case occurred on an interstate highway and the speed was documented by eyewitnesses and was not in dispute. Using the speed of the vehicle and the drop height of the vault, the length of the vault can be calculated.  The documentation of the start of the tire marks also defined the lateral movement of the vehicle.  With the vault length and the lateral distance, the departure angle is a simple trigonometric calculation.

The departure angle as calculated from the manual police measurements was over 20 degrees and using the vault calculation just under 15 degrees.  The lower departure angle put the tire marks in a location more consistent with the survey information.  The lower angle also makes it easier to argue that a guard rail would make a difference.

**Departure Angle, Vault, Roadway Departure**

## BS6  Taking Sexual Assault Response Teams To The Next Level: The California SART Report

*William Green, MD*, California Clinical Forensic Medical Training Center, University of California Davis Medical Center, 3671 Business Drive, Sacramento, California 95820; Marilyn Peterson, MSW, MPA*, CAARE Diagnostic and Treatment Center, 3300 Stockton Boulevard, Sacramento, CA 95820; and Brooke Allison, MA*, c/o CA Clinical Forensic Medical Training Center, 3671 Business Drive, Sacramento, CA 95820*

After attending this presentation, attendees will understand the most current research findings on key elements and promising practices for SART (Sexual Assault Response Team) effectiveness. The presentation will also include policy and legislative recommendations to enhance SARTs through state and local advocacy.

This presentation will impact the forensic science community by providing attendees with a copy of the California SART Report which, when combined with the presentation and discussion, will provide attendees with a set of tools for advancing SARTs to the next level.

Presentation information derived from the California SART Report is based on most comprehensive research ever conducted on how SARTs operate, what works best, what's needed for increased effectiveness, and how to take the field to the next level of service and sustainability.

The report data derived from a three-pronged methodological approach, combined a national review of extant literature and research findings, a statewide (58-county) electronic survey, and in-depth interviews with SARTs in the field. The result is the largest, most comprehensive database on SARTs in the United States with the analysis of findings from 308 survey respondents representing every county in California and in-depth site visits with 19 SARTs from across California. The diversity and depth of the data collected make the findings eminently pertinent and transferable to the experiences and needs of SARTs in the other 49 states.

Based on the report's comprehensive research, the presentation will provide participants with a thorough overview of key SART elements, promising practices, and case study examples from the field that demonstrate promising practice in action.  Both the roles of the various disciplines and agency partners, and the essential organizational infrastructure and institutional practices required for SART operational effectiveness will be covered. These will be discussed within the context

of how to apply the findings and recommendations for the best local effect. These questions and several other highly controversial and provocative issues relating to the Memorial Hospital deaths will be discussed and should be of significant relevance and pragmatic concern to forensic scientists, attorneys, and many other professionals throughout the world.

**SART Research, Promising Practices, Advancing SARTs**

## BS7 Broken Bones, Bites, Taphonomy, and Tool Marks: Getting More From Traumatized Bones

*Steven A. Symes, PhD\*, Mercyhurst Archaeological Inst, Mercyhurst College, 501 East 38th Street, Erie, PA 16546-0001*

The goal of this presentation is to inform attendees about New Era Anthropology and the pursuit of indicators
contributing to accurate assessments of cause and manner of death.

This presentation will impact the forensic science community by illustrating new techniques used to analyze victims of violent deaths, while highlighting common mistakes made.

A decomposed body is found with bone that is scored, scraped, and scratched. Forensic anthropologists and pathologists face the same age-old questions: Killers or Critters? Could the marks spell malicious intent by a perpetrator, or might they simply be postmortem carnivore chew marks? Is today's forensic scientist equipped to determine forensic significance from the marks or fractures left on bone? And, if so, why is a tooth not a knife?

For the record, traumatized bone can accurately record violence, but unfortunately, precise interpretation of violence expressed on bone is never an easy task. Further, erroneous interpretations may result in severe repercussions, especially when those interpretations misidentify taphonomic influences as indicators of cause of death.

This presentation discusses the triumphs and pitfalls of bone trauma analysis in the following major areas, using common quotes associated with the trauma as illustration:

Sharp Force Trauma

*Yes, the body has been dismembered, but there are no diagnostic characteristics.*

*Duplication of shallow cut mark features... or 'hesitation marks,' is common in dismemberment. . .*

*If I can see it, I can measure it. . .*

Blunt Force Trauma

*Inbending cranial bone creates outbending at different places on the skull. . .*

*The shape of the wound indicates the shape of the tool. . .*

Bones Burned

*Bodies burned in fires show few patterns, with the exception that skulls explode when heated. . .*

*[Pamela Mayne accurately states in 1997: There is no satisfactory method available, based on visual observation of fractures, to differentiate the condition of bone prior to cremation.]*

Ballistic Trauma

*Wounds from arrows, guns, and slingshots can all be classified and interpreted similarly since each represents 'projectile' trauma...*

*Bouncing bullets. . . rattling around in there?*

*Confusion with ballistics; what confusion? Hell, now I'm confused.*

An advanced knowledge of postmortem influences on human remains, and an understanding of traumatic insult to bone and related tissues, equip today's specialists with the necessary tools to examine human remains and, ultimately, interpret criminal behavior.

"Getting More from Traumatized Bones" should enable the forensic scientist to resurrect the victim's story with newfound clarity and cast new light on the victim's demise.

**Bone Trauma, Taphonomy, Tool Marks**

## L1 When You're In Hell, Don't Screw With the Guy Holding the Pitchfork

*Larry Pozner, JD\*, Reilly Pozner LLP, The Kittredge Building, 511 Sixteenth Street, Suite 700, Denver, CO 80202*

Expert witnesses and other professionals who testify in court are bombarded with offers of high-dollar seminars purporting to teach "How to Be a Fatal Expert Witness in Court" or "Beating the Best: Making Fools of Lawyers Who Dare to Cross-Examine You." After attending this presentation, attendees will have the opportunity to learn how attorneys prepare for and execute cross-examinations of expert witnesses.

This presentation will impact the forensic science community by providing attendees with an expanded skill set for preparing to deal with expert testimony at trial.

Cross-examination is a process intended to assist in exposing the whole truth of a witness's relevant knowledge to the finder of fact in a case. If science embodies broadly accepted concepts and practices, the expert witness should testify within well-established, known limits.

The attorney engaging in cross-examination should be familiar with professional standards and practices in the specific area of expertise professed by the expert who will be examined. Exposure of departures from broadly recognized scientific norms of practice or knowledge can be fatal to the witness. Where scientific integrity is shown to be compromised, exposure can be fatal to the witness. Cross-examination of the expert is only one component of neutralizing that expert's exclusive value to the other side. It is also the diametric opposite force to the "hired gun" or "advocate" witness. In extreme instances, it is the embodiment of "you can run, but you can't hide."

This talk will include illustrations of cross examinations in which the expert is more properly the subject of a book such as The Naked and the Dead. The forensic scientist who testifies should leave the presentation with a greater appreciation of the limits of their ability to out-maneuver opposing counsel at trial, and a broader view of the lawyer's use of the powerful tool of cross-examination in dealing with expert testimony.

**Cross-Examination, Trial Preparation, Trials**

## L2 Neuroscience and the Law: Forensic Applications of Cerebral Single Photon Computed Tomography in Mild Traumatic Brain Injury

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After attending this presentation, participants will become familiar with the process whereby emerging medical technologies may be assessed for admissibility. Specifically, attendees will learn about the current state of evidence surrounding the application of cerebral SPECT imaging to mild Traumatic Brain Injury and how to appropriately utilize such evidence in forensic contexts.

This presentation will impact the forensic science community by illustrating the challenges surrounding the introduction of emerging medical technologies to forensic application. The appropriate use of cerebral SPECT imaging in mild Traumatic Brain Injury litigation is reviewed.

The rapid rate of development in the neurosciences has broad implications, not only for medicine and patients, but for society and humanity at large. Many believe that as the secrets underlying brain function are gradually unraveled, the ability to comprehend, anticipate, and ultimately alter human behaviors will be realized. Such notions have profound implications, particularly when basic assumptions about human thought and behavior, like free will and responsibility, are challenged. Unfortunately, the excitement surrounding these scientific developments, and their potential seductive powers, has resulted in many instances of premature and questionable applications of neuroscience to the law. In this context, a process whereby emerging technologies may be carefully considered in terms of scientific support and the applicable rules of evidence is essential.

Traumatic Brain Injury (TBI) is a substantial source of mortality and morbidity world-wide. Although the majority of such injuries are relatively mild, accurate diagnosis and prognostication after mild TBI is challenging. These issues are complicated further when considered in medicolegal contexts, and particularly civil litigation. Cerebral Single Photon Computed Tomography (SPECT) imaging may identify functional brain abnormalities following mild TBI, and some parties may seek to introduce SPECT findings as evidence in legal proceedings related to TBI. The frequency of mild TBI, the increasing clinical availability and application of SPECT, and a litigious environment unite to yield an atmosphere in which the introduction of evidence involving the interpretation of SPECT images is inevitable. However, independent reviews of the rules of evidence relevant to the introduction of SPECT in such cases have not previously been published. The application of SPECT to mild TBI is presented as an example of the process whereby emerging medical technologies may be evaluated for admissibility into courts of law.

A Medline and PsycInfo database search for the years 1965 to 2006 anchored to TBI and SPECT is performed, and peer-reviewed practice parameters regarding SPECT imaging are reviewed. Rules of evidence based on Frye vs. United States, Daubert vs. Merrell Dow Pharmaceuticals, the Federal Rules of Evidence, and General Electric vs. Joiner are used to evaluate the suitability of SPECT imaging in mild TBI litigation. The theory behind SPECT abnormalities after TBI is a subject of active investigation and findings from such investigations have been subjected to peer-review and publication. However, there remain substantial uncertainties regarding the rates of error and also disagreements regarding the methods for performing clinical cerebral SPECT imaging in this context. While standards for the performance, interpretation, and ethical reporting of SPECT images exist, the present literature suggests that these requirements are infrequently met in research and routine clinical applications. Additionally, the usefulness of cerebral SPECT imaging in the evaluation of TBI is not generally accepted in the scientific community.

This analysis of the suitability of cerebral SPECT imaging in mild TBI casts serious doubt on the evidentiary usefulness and appropriateness of this technology at this time. Ethical testimony on cerebral SPECT imaging in mild TBI requires open acknowledgement of the limitations surrounding technical quality, clinical data, evidentiary support in the literature, and the unclear relationships between SPECT imaging patterns and their etiologies or clinical correlations. While clinicians and scientists are gaining experience with SPECT in mild TBI, the level of understanding surrounding the injured brain and this relatively new technology have not united to a degree sufficient to establish causal relationships between cerebral SPECT imaging findings and mild TBI or its

neurobehavioral sequelae. In light of the need to regard cerebral SPECT as a secondary line of evidence, it would appear at best to be a superfluous evidentiary device whose appropriate forensic purpose is to augment the communication of diagnostic impressions derived from other sources of clinical evidence through the presentation of colorful and easily understood "brain images" to participants in legal proceedings. Expert witnesses should acknowledge this fact; when they fail to do so, officers of the court should require from them such an acknowledgement. Accordingly, the use of SPECT imaging in the context of mild TBI litigation is not recommended.

**SPECT, Traumatic Brain Injury, Neuroscince**

## W1 Ethics in the Practice of Forensic Science

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After attending this workshop, participants will learn: (1) the relationship between science, law, and law enforcement; (2) how science utilizes ethics; (3) the ethical issues facing forensic scientists; (4) some of the major ethical issues affecting forensic scientists; and (5) what ethical standards are in place for forensic scientists.

This workshop will impact the forensic science community by demonstrating how ethics is an understudied, yet significant topic when it comes to the field of forensic science.

Ethics is an understudied, yet significant topic when it comes to the field of forensic science. Although people may think of ethics as a personal matter, it also includes professional and public issues. Proper ethical behavior is required by scientists making complex decisions about the interpretation of data, about which problems to pursue, and about when to conclude an experiment, all which help to improve the quality of forensic science. Through investigation into the ethics of science, health, business, and research West Virginia University's Forensic Science Initiative has identified which ethical issues are most prevalent in the forensic science community. Important skills gained by studying ethics include improved ethical awareness, knowledge of relevant standards (AAFS, IAI, ASCLD, etc.), skill in ethical decision making, and appropriate ethical actions. This workshop will provide attendees with an overview of ethics as it pertains to forensic science.

The major areas of concern within forensic science include falsification, fabrication, and misuse of resources. Another common concern among many fields, including forensic science, is the misrepresentation of credentials. The issue of misrepresented credentials is prevalent in the presentation of expert testimony. It is shown that people often over look seemingly smaller ethical issues, such as padding resumes and travel expenses. These issues are closely observed to determine the potential impact on the forensic science community.

This workshop has been developed in response to the lack of formal ethics education specific to forensic science. While it includes many "basics," the workshop relates those ideas to the forensic science profession. Participants will learn and discuss how ethics can affect all forensic service providers. To understand forensic-specific ethics, it is important to look at the interactions between the cultures of science, law, research, and law enforcement. This presentation will provide attendee's understanding of: (1) how law enforcement approaches ethics; (2) ethical concerns of the expert witness; (3) why science is naturally an ethical field; (4) conflicts of interest and other potential problems; and (5) where people get into ethical turmoil

This workshop will be broken into lecture and interactive group activities. Attendees are given the opportunity to interact and discuss ethical situations that have taken place within the forensic science community. Attendees will be presented with scenarios and the ethical considerations involved with each. The attendees will provide insight from their work environments and represent the "real-world" of ethics in forensic science. Participants should be open to discuss and debate, while keeping an open-mind and a positive environment.

**Ethics, Professionalism, Standards**

## W2 New Insight into Asphyxia by Hanging: From Basic Hanging Deaths and Autoerotic Asphyxial Fatalities to Advanced Pathophysiology of Human Hanging

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After attending this workshop, attendees will become familiar with basic hanging deaths and autoerotic fatalities by asphyxia, specifically those that involved hanging, starting from basic crime scene investigation, reconstruction of the event, and autopsy findings. Attendees will also be given the opportunity to gain valuable new knowledge on the pathophysiology of human hanging and understand the impact of this knowledge on crime scene investigation, autopsy findings and court testimony.

This workshop will impact the forensic science community by providing a review of current knowledge and presenting recent developments on the understanding of asphyxia by hanging.

**Review of current knowledge in asphyxia by hanging:** Asphyxia by hanging is a form of asphyxia secondary to compression or constriction of neck structures by a ligature tightened by the weight of the body. Body suspension can be complete or incomplete. Death is caused by compression of the blood vessels of the neck and/or obstruction of the airway. The amount of pressure to necessary to compress the jugular veins is 4.4 lbs.; the carotid arteries, 11 lbs.; the trachea, 33 lbs.; and the vertebral arteries 66 lbs. Virtually all hangings are suicidal, though accidents are sometimes encountered, particularly in children, mentally or physically disabled patients, or in autoerotic context. True homicidal hangings and simulated homicidal hangings have also been reported. Basic knowledge on scene investigation including a presentation of interesting case histories and autopsy findings will be further discussed, to include current concepts on autoerotic asphyxia.

**New developments in asphyxia by hanging:** The Working Group on Human Asphyxia was formed in 2006, at the 58th Meeting of the AAFS in Seattle. This group's main objective is to regroup filmed hangings in order to give new insights into the pathophysiology of human hanging. So far, a total of eight filmed hangings from three different countries (Canada, Switzerland, and United-States) were analyzed: two filmed suicides and six autoerotic deaths. Hangings were of different types: free hanging, hangings with feet on the ground, hanging kneeling, and hanging almost lying face-down. The hanging ligatures also varied widely, from cloth band to ropes with or without padding, and electric cords. All victims were adult males.

In this advanced part of the workshop, seven of the eight filmed hangings will be presented and discussed. Respiratory and movement responses to asphyxia by hanging will be described in details. With the time 0 representing the onset of hanging, rapid loss of consciousness was observed (at 8 – 18 seconds), closely followed by appearance of convulsions (at 10 – 19 seconds) in all cases. A complex pattern of decerebration and decortication rigidity was then observed in all cases. Last isolated muscle movement occurred between 1 minute-2 seconds and 7 minutes-31 seconds. As for respiratory responses, onset of very deep respiratory attempts was observed between 13 and 24 seconds and

last attempt between 1 minute-02 seconds and 2 minute-05 seconds. Results will be compared to previous animal studies.

Finally, limb bruises found in hanging victims will be described in correlation with observed body movement in filmed hangings. Suicidal hanging of 207 cases were retrospectively reviewed and compared to 45 homicidal non-hanging strangulation victims. Limb bruises on hanging victims were generally located on the posterior upper limb or the anterior lower limbs, whereas strangulation victims did not display this preferential bruises concentration. Bruises distribution will be discussed in relation to decortication and decerebration rigidities.

**Asphyxia, Hanging, Autoerotic Fatalities**

# W3 They're Alive! Breathing New Life Into the Investigation and Prosecution of Cold Case Homicides

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Time has traditionally been an enemy in homicide investigation. In cold case homicide investigation; however, it may become an ally. Upon completion of this workshop, the participant should be able to recognize the strengths and weaknesses present in cold cases, gain awareness of investigative methods utilized in these cases, and further understand the application of advances in technology and changes in relationships as means and methods to exploit the passage of time in solving cold case homicides and identification of victim-witness issues.

Criminal homicide is the ultimate personal crime and for almost five decades the nation has been experiencing an almost continuous decline in the solution of murders. While the FBI and others annually compile and publish murder related statistics for respective years, the exact number of unsolved homicides is unknown as the data is retained within the individual law enforcement agencies responsible for their solution. In recent years, many law enforcement agencies have been reinvestigating long unsolved cases to hold those to account who thought they got away with murder. This presentation will impact the forensic science community by enhancing awareness and understanding of the problem, encourage reopening of unsolved cold cases, and offer potential solution methodologies to those who may become involved in the forensic, investigative, or legal and victim-witness environment of cold case homicides.

In the past two decades, the criminal investigative environment has experienced a paradigm shift due to significant advances in the means and methods of crime detection and suspect identification. After attending this presentation, attendees will learn how teamwork between law enforcement, the forensic laboratory, and prosecutors has resolved previously investigated but unsolved "cold case" homicides. Cold case investigations present special challenges not present in the investigation of current "hot" homicides, and investigative methodologies may involve a multitude of forensic disciplines not normally encountered. As a result of these cumulative efforts, perpetrators who thought they got away with murder are now held accountable for their crime and what might be considered as "justice" for the victims and their families may finally be attained.

Through examination of the history of cold cases and the homicide environment, participants will learn how and why many homicide cases went unsolved over the years. Time, once considered an enemy in homicide investigation, may now become a friend as changes in technology and focus on changes in relationships have allowed modern investigators the opportunity to exploit the passage of time and make it investigation-friendly. This presentation will illuminate this transformation within the investigative landscape.

Participants will learn how cases are reopened, and how those with the most potential for solution are selected for re-investigation. This presentation will explore critical issues such as file and evidence recovery, legal considerations, and the value and use of behavioral analysis in the investigation process. Additionally, this presentation will examine crime assessment as a method to capture evidence, and address and illustrate a protocol for evaluating the presence and absence of crime scene evidence and measuring it against known standards of crime typologies.

In 1983, a young mother was kidnapped and murdered in Los Angeles. Despite the best efforts of detectives and the laboratory, this case went unsolved for 20 years. Attendees will follow the case from its origins and will learn firsthand from the experienced detectives and prosecutor how this case was finally solved and prosecuted two decades later. This case study will vividly illustrate the successful integration of investigative, forensic, and prosecutorial methodologies and strategies.

Attendees of this workshop can expect to gain a better understanding of the cold case investigative and prosecution environment, and the fundamental issues and obstacles that confront those charged with solving homicides that, if they had been easy, would have been solved years before.

**Cold Case, Homicide, Behavioral Analysis**

# W4 Microscopical Thinking and Trace Evidence

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After attending this presentation, attendees will understand that trace evidence approaches and microscopical thinking have a wide applicability in criminalistics.

This presentation will impact the forensic science community by illustrating how microscopical thinking can have a positive impact on trace evidence analysis.

This workshop is designed to provide the participants with a broad perspective concerning the role of microscopy and that of a *generalized microscopical*, or *trace evidence*, approach to the problems of physical evidence assessment and interpretation in complex cases. It is expected that those participating in the workshop will possess a basic theoretical understanding of polarized light microscopy, as well as a modicum of practical experience with the use of the polarized light microscope. While fired bullets and other ammunition will be used to illustrate the concepts presented, the scope of this workshop applies to all types of forensic evidence. The point will be made that trace evidence approaches have an extraordinarily wide applicability in criminalistics. Furthermore, the applications of this approach to casework transcend the dimensional constraints of the microscopic domain, since this process is not limited only to material transfers.

**Trace Evidence, Criminalistics, Microscopy**

## W5    Pediatric Forensic Medicine

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The goal of this workshop is to present new information and reinforce existing knowledge regarding deaths occurring in the pediatric age group other than those due to abusive head trauma of the shaken-impact type. Upon completion of this workshop, the participant will have a better understanding of investigation of these deaths including those which occur in utero, related to birth injury, due to asphyxia (whether accidental or homicidal with recognition of unsafe sleep environments), and those due to natural diseases including those which are difficult to diagnose at routine autopsy alone (metabolic disorders and cardiac channelopathies). In addition, the participant will have an understanding of the ways that fractures can be demonstrated and evaluated postmortem. The participant will have a better understanding of more subtle forms of fatal abuse including neglect. Understanding the differences between pediatric patients and adults regarding metabolism of drugs and the significance of these differences in prescribing and hopefully in preventing death is also a goal. Finally the participant will be able to recognize the importance of adequate death investigation in cases of more subtle forms of abuse as well as in potentially heritable natural disease in the hope of preventing future deaths. The participant will also be aware of possible screening programs for some inherited disorders at birth and prior to participation in athletics in an effort to prevent other deaths.

This presentation will impact the forensic science community by focusing attention on the area of pediatric forensic medicine and the importance of thorough investigation of these deaths not only for the appropriate classification of the cause and manner of death, but for complete documentation of injuries and natural diseases which may provide information about the circumstances surrounding the death and possible contributory mechanisms. In addition, recognition of risk factors in some cases may aid in the prevention of future deaths. These include factors which may result in birth trauma or fetal death, recognition of unsafe environments which place a child in danger of accidental death including unsafe sleep environments, recognition of subtle forms of fatal abuse, and knowledge of natural disease with hereditary components. Recognition of these factors may lead to policy changes designed to positively impact public health by preventing future injuries and deaths.

Deaths in the pediatric age group (less than 18 years of age) represent a significant number of the cases that any death investigation system is required to accept and investigate. Many of these are due to intentionally inflicted injury at the hands of another person (homicide) often due to head injury. While much research and many lectures have been dedicated to abusive head injury particularly of the shaken-impact type, these cases are but one of the many types which are placed in the hands of the forensic pathologist and death investigator for proper evaluation and certification. Deaths in the pediatric age group begin with birth and continue through late adolescence until adulthood. This workshop will address a variety of these deaths beginning with fetal deaths and birth trauma, followed by a discussion of deaths related to asphyxia with topics including unsafe sleep environments, accidental asphyxia of various causes and homicidal asphyxia. More overt evidence of child abuse will be addressed in lectures on burns and cutaneous evidence of injury as well as postmortem detection and evaluation of fractures using multiple methods including radiography, gross examination and histology. Discussion of deaths due to neglect and more subtle forms of abuse will be followed by a review of natural diseases which may result in death. Some natural causes of death may be difficult or impossible to diagnose by autopsy alone such as various metabolic disorders and inherited arrhythmias; however, since many are inherited recognition is vitally important. Postmortem testing for some of these disorders is now available although costly. Discussion of these disorders particularly the cardiac channelopathies including the importance of recognition will be included in this program. Possible alternatives to postmortem testing, counseling recommendations and the increasing role of pre-participation screening in athletics will also be briefly addressed. Some of these inherited arrhythmias may be simulated or exacerbated by certain drugs. Drug related deaths in the pediatric age group are potentially preventable, as many of the aforementioned deaths are, and the program will conclude with a lecture on pediatric toxicology. Emphasis on the difference between children and adults regarding drug metabolism, etc., may provide insight into some cases of drug-related pediatric deaths and possible prevention of these in the future.

**Pediatric Forensic Medicine, Asphyxia, Toxicology**

## W6    So You Think You Know Digital Imaging? SWGIT Advice To All AAFS Disciplines

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After attending this workshop, attendees will learn best practices for taking, processing and archiving digital images in the forensic sciences. At the end of this workshop, attendees will be better prepared to ensure that his or her photographic evidence is suitable for further analysis and admissibility in court.

Digital imaging and image processing are fundamental to forensic science today. Despite this, many forensic scientists lack a basic understanding of best practices for photography and image processing. Failure to follow best practices could lead to incomplete or incorrect analytical results, as well as the exclusion of evidence. This workshop will impact the forensic science community by improving the quality of photographic evidence in the forensic sciences.

The object of this workshop is to provide forensic scientists with practical guidance on how to take, process, analyze, and preserve digital images so that they will be admitted in court. Practical guidance will be provided on such issues as "What sort of camera or scanner should I use?", "Should I shoot images in RAW or JPEG?", "How should I process my images?", and "Should I preserve my images on compact disc, on paper, or on a hard drive?" Attendees will learn about the steps needed in documenting the photographic process. Forensic scientists and lawyers will learn about the myths and reality regarding digital imaging and will also learn when an image expert or subject matter experts should be called.

An overview of basic photographic processes and procedures necessary to obtain photographs that are accurate and of high quality,

regardless of the subject will be provided. This introductory session will address questions regarding the selection of equipment, including cameras, lenses, and lighting. Operational issues will also be addressed including if and when the photographer should record images using "RAW" formats and JPEG compression. Best practices regarding how to save and store original digital image files, including the media on which to save them, will be discussed.

Issues of spatial resolution and color accuracy will be addressed, to include methodology for determining the "practical" resolution of his digital camera. Resolution and color accuracy involve more than just the selection of a camera or scanner, so the lessons learned from this session should enable any forensic scientist to determine the practical resolution of his or her entire imaging system.

A series of lectures aimed at imaging and image processing for specific forensic disciplines, including latent print/laboratory photography, questioned documents, forensic pathology, forensic odontology, and forensic anthropology. General procedures will be addressed, as well as special challenges, in each of these disciplines. The presentation on forensic odontology will include a discussion of a new technique for comparing a bite mark to a dental model using 3-D imaging techniques; the forensic anthropology presentation will focus on biometrics of the face and ears, and will include the proper technique for photographing faces so that the resulting images will be suitable for use with facial recognition applications.

Image processing, image integrity and archiving will be addresses, a detailed rebuttal of common myths regarding digital imaging and digital image processing will be presented, as well as a description of relevant case law. Attendees will learn about some of the most common image processing procedures used to improve the quality of their image evidence, as well as how to best document the use of those procedures. Steps to preserve images and demonstrate the integrity of these files will likewise be discussed. Students will be provided with background information necessary to ensure that his or her image evidence will be accepted in court.

**Digital Imaging, Image Processing, SWGIT**

analysis methods, and the association of counterfeit documents to one another and to criminal organizations.

This presentation will impact the forensic science community by exposing attendees to modern security features and methods used to forensically link multiple documents that can be used to conduct examinations and render conclusions.

Counterfeit documents pose some of the greatest threats to modern society in terms of physical security, the integrity of our financial system, and to our personal identities. With the escalating value of personal and financial data to a counterfeiter, along with the rapid evolution of technology and the increased amount of information available via the internet, corrupt individuals are becoming increasingly motivated to perpetrate these crimes for the purpose of financial gain. Even more of an impact on national security, the attacks on September 11, 2001 have resulted in an effort to increase the integrity of government issued travel documents such as passports and drivers licenses. As a result, the Real ID Act was mandated by the federal government in 2005 requiring authentication and issuance standards for state identity cards if they are to be officially accepted at federal government sites such as airports and certain office buildings. Although the submission of counterfeit documents for forensic analyses usually takes place after the documents have been used and the crime has been committed, this function serves a particularly important role in combating fraud. Forensic examinations can provide investigative clues regarding the perpetrator(s), how the documents were constructed, if they are associated with other counterfeits, and if they were produced using materials seized from a suspect(s). As well, the forensic document examiner is sometimes called upon to provide expert testimony in a court of law in order to convey the findings of an examination. Indeed, this is a critical stage in the legal process and therefore, it is incumbent upon the document examiner to have a solid foundation in the examination of counterfeit materials so that he or she can provide unbiased and accurate information to the courts.

**Counterfeit Documents, Security Features, Link Analysis**

## W7 Security Documents Before and After the Crime: REAL ID, Physical and Electronic Security Features, Developments in Commercial Printing Technology, and an Introduction to Counterfeit Link Analysis

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After attending this presentation, attendees will receive: (1) an update on the REAL ID Act of 2005 and the impact on the future of forensic document examinations, (2) technical information regarding the latest security features being incorporated into documents, including electronic media such as RFID technology, digital watermarking, and biometrics, (3) developments in printing technology and the complexity of contemporary printing process identification, and (4) counterfeit link

## W8 Solid Phase Extraction in Forensic Science-Principles and Applications

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The goals of this workshop are to teach the basic science and chemistry of SPE, give insight into how to develop SPE methods for compounds of interest, and review some of the newer methods for extraction of specific compounds.

This presentation will impact the forensic science community by providing a great review for the experienced toxicologists and a highly informative session for the beginner.

This workshop will give a comprehensive view of SPE in forensic toxicology providing a discussion of the history, development, and evolution of conventional and new techniques involving SPE. The theory and use of theory to develop methods will also be discussed. In addition, an in depth discussion of silica gel and the chemistries associated with bonded phases will be presented including problems that arise if the chemistry is not done correctly. A description of all available sorbent types and their chemistries will be presented.

Presentation of mechanisms and how to use them to improve selectivity and recovery will be included. Adsorption, reverse phase and ion exchange will be discussed in detail. A review of equilibrium drugs in solution will be conducted along with a discussion of pKa and log P and how this information is useful in doing method development for drug extractions will be related.

Attendees will be taught step by step method development for a variety of drug classes. Each step in the method development sequence will be discussed and the details of what to do to make your methods successful will be reiterated. Furthermore, several philosophical approaches to method development will be discussed in addition to the pros and cons of each. Troubleshooting methods will be presented and attendees will be taught where to look for problems in the method development sequence and how to systematically eliminate problem sources.

An extensive series of applications will be reviewed and each step in the method will be reviewed. The classes of drugs discussed will be barbiturates, opiates, THC's amphetamines, PCP, cocaine, LSD, quaternary amines, methyl malonic acid, gabapentin, ETG, benzodiezapiens, and many others. The applications will be presented in a way which will help the toxicologist understand the importance of adjusting the extraction conditions to make optimum use of the correct mechanisms to optimize the extraction. Along with the normal extraction techniques, methods such as benzodiazipines will be evaluated using more than one mechanism showing the results of the different approaches and what can be achieved or missed by choosing a different mechanism.

A discussion of challenging matrices will be included. All of the common matrices such as urine, serum, plasma, and blood will be discussed along with a list of not so common matrices such as hair, sweat, sebum, orcular fluid, maggots, and others. A new cutting end technology describing the use of oral swabs in postmortem forensic toxicology will be presented.

The use of robotics in SPE will be introduced. A method will be discussed and demonstrated with actual data.

A section of LCMS in forensic toxicology will be included in this presentation to introduce the forensic scientist to this new and powerful technique which is becoming a popular technique very quickly.

A review of derivatization reagents will be the final part of this presentation. It will include silylation, acylation, methylation, and other frequently used techniques. Each class of compounds, along with their advantages and disadvantages, will be discussed and listed.

Resources and references will be included in this presentation which will provide the scientist a variety of places to go to seek additional information.

**SPE, Forensic Toxicology, Method Development**

## W10    Digital Forensics in Large Scale Cases

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After attending this presentation, participants will gain insight into ways in which digital and multimedia forensics can be applied in large-scale case situations.

This presentation will impact the forensic science community by demonstrating how digital forensic techniques can be used in cases involving large numbers of devices to be examined or large numbers of individuals impacted, particularly where time and resource pressures are significant.

The field of digital forensics has evolved from cases that often involved the examination of one or two devices, to much larger scale cases involving tens, hundreds, or even thousands of devices, all of which must be properly accounted for, imaged, examined, and analyzed. Other cases may involve a limited number of devices, but may impact hundreds of thousands of individuals.

Attendees will learn how these problems have been addressed by practitioners and see how a combination of adapting standard practices along with innovative extensions to those practices have made the handling of large cases, if not easy, at least possible. This workshop will consist of the following elements:

*Introduction to Large Scale Case Handling: A*n overview of the topic with some historic background on the evolution of the size and scope of digital and multimedia cases will be presented.

*Application of Digital Forensics to Large-Scale Identity Theft Incidents:* With identity theft becoming the number one white collar crime in America, understanding how – and whether – identity theft occurred will be reported. A series of case studies demonstrating the range of outcomes to be expected in such matters will be presented.

*Investigating Microsoft® SQL Servers in the Event of Unauthorized Data Access or Compromise: A* more detailed look at how features of SQL can be leveraged to extract valuable information that is often unavailable anywhere else and can aid investigators attempting to understand a database compromise incident will be presented.

*Justice Delayed is Justice Denied: Proposals for Expediting Forensic Examinations of Digital Evidence:* Increasing case sizes and case volumes have resulted in unacceptable backlogs in processing of digital forensic evidence. A tiered strategy for performing such work and discussions of updating policies and protocols in digital forensic laboratories to deal with this growing challenge will be presented.

*Confronting the Reality of Large Data Set Analysis in the Department of Defense:* How DoD's ASCLD-LAB accredited facilities have handled the rapid growth in workload will be reported. Combining case studies and discussion, this session will provide some practical advice of use to all digital evidence practitioners.

**Digital Forensics, Case Management, Forensic Laboratory Management**

## W11    Integrated Microfluidics for Forensic Applications

*Joan M. Bienvenue, PhD\*, 8604 General Sykes Circle, Fredericksburg, VA 22407; Robert C. Giles, PhD\*, Orchid Cellmark, Incorporated, 13988 Diplomat Drive, Suite 100, Farmers Branch, TX 75234; James P. Landers, PhD\*, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904; and Suzanne C. Bell, PhD\*, West Virginia University, 1600 University Avenue, Oglebay Hall Room 208, Morgantown, WV 26506-6121*

The objective of this workshop is to educate a broad audience on the principles of microfluidic systems and their incorporation and application in the forensic sciences.

This presentation will impact the forensic community by providing a forum for in depth discussion of microfluidics, an opportunity to educate a larger audience with a focused, intensive presentation, and an occasion to discuss the practicalities and possibilities of this emerging technology.

The development of microscale analyses and the micro-total analysis system (μTAS) concept for a lab-on-a-chip for a variety of applications that will impact forensic science is now rapidly becoming a reality. Microfluidic technology stands to revolutionize the way not only

forensic genetic analysis is accomplished, but also drug analysis, toxicology, explosive detection, poisons, and other small molecules. Incorporating sample pre-treatment steps such as purification, and PCR amplification, with microchip electrophoresis in multi-purpose, multi-functional devices capable of total, rapid, and automated analysis for a wide variety of forensic applications is currently the focus of much research effort. A fully-integrated, microchip capable of performing the steps normally carried out at the bench would not only reduce the time required to perform these tasks, but would also eliminate user intervention and potential sources of contamination, preserving more of the sample for future analysis. Optimization of these devices for forensic analyses, however, presents a distinctive set of challenges.

Due to the multi-step nature of many forensic analysis processes, careful consideration must be given to solution compatibility, sample size, and fluidic interfacing in order to seamlessly integrate these technologies, both for DNA analysis, as well as the other applications highlighted above. As commercialization of microfluidic systems nears fruition, the forensic community is provided with the unique opportunity to shape the final design of what promises to be a revolutionary change to the way these analyses are carried out.

This workshop will provide the attendee with a comprehensive overview of the current state of development of microfluidics for forensic analyses, a foundation for understanding the principles of microfluidics and how current processing methodologies are being translated to the microscale. While the workshop will focus on genetic applications of microfluidics, research efforts in other forensic disciplines will also be presented, to provide the attendee with a broad understanding of the principles and diversity of microfluidic systems.

Further, the role of microfluidic systems and practical considerations for their application in forensics labs and in portable genetic analysis systems will be discussed. The attendee will also gain an appreciation of this new technology, its limitations, and the unlimited potential of its application and use in the forensic laboratory. Concerns and criticisms of this new technology will be addressed from the view of the forensic analyst and an open forum discussion will be included. Finally, a view of the future of advanced microscale analytical systems for these applications and their impact on the community will be highlighted.

**Microchips, DNA, drug analysis**

## W12    Quality Assurance in Human Identification

*Vincent J. Sava, MA*, John E. Byrd, PhD*, and Thomas D. Holland, PhD, JPAC, Central Identification Lab, 310 Worchester Avenue, Building 45, Hickam AFB, HI 96853*

After attending this presentation, attendees should be able to understand the basic quality assurance principles and measures applicable to human identification. Participants will learn the unique challenges faced by professionals involved in human identification when striving to have their facilities, procedures, and casework meet the standards demanded by the criminal justice and medical-legal systems. Attendees should be able to utilize the material presented to formulate a quality assurance program for their organization.

This presentation will impact the forensic community by demonstrating how quality assurance in forensic laboratories and forensic programs has lead to objective and measurable standards of performance that ultimately strengthen and elevate the forensic profession as a whole.

Quality assurance programs in forensic laboratories and activities have been a growing trend over the past decade. Since 1999 the Joint POW/MIA Accounting Command, Central Identification Laboratory (JPAC-CIL) has implemented a stringent quality assurance program to ensure the scientific integrity of its casework. The CIL's quality assurance program ultimately led to the Laboratory's accreditation by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB) in 2003—the first forensic skeletal identification laboratory to be so credentialed. In 2008 the CIL was re-accredited under the ASCLD-LAB *International Program* using ISO 17025 Criteria

The goal of this workshop is to introduce the attendee to the CIL's Quality Assurance Program and to convey the lessons learned resulting from its implementation and growth. A video overview of the JPAC CIL is presented followed by an overview of its quality assurance program. In the latter, the concept of the scientific integrity of the laboratory is discussed followed by a summary of the "Surety" model of quality assurance.

The participants will become familiar with each measure that comprises the surety model of quality assurance. The importance of integrating and synchronizing all of the surety measures discussed during the workshop will be continually stressed. Infrastructure and support considerations necessary for a successful quality assurance program are also presented. Surety measures addressed include, but are not limited to: (1) desired qualities of a laboratory manual and other vital documentation and their control, (2) adequacy and safety of laboratory facilities, (3) policies and procedures conducive to a positive work environment, (4) evidence management and security, and (5) training and professional development.

Gathering and interpreting evidence where quality assurance in field operations and trace evidence analysis is discussed. The surety measures directly related to casework – the peer review process, validation of technical procedures, case file management, analytical notes, and documentation – are presented for consideration.

Quality assurance procedures and programs are ineffective in the absence of monitoring, enforcement, and corrective action. These are accomplished through a myriad of surety measures including proficiency testing, review of court testimony, audits, annual reports, and corrective action policies, which are presented.

Attendees will become acquainted with the problems that hindered, and the processes that led to, the accreditation and re-accreditation of the JPAC CIL. Surety assistance programs offered by the CIL will be discussed in the event an attendee's organization desires assistance with their surety programs or accreditation efforts. Additionally, the contributions, to date, of the Scientific Working Group in Forensic Anthropology (SWGANTH) to the human identification profession will be briefly discussed.

**Quality Assurance, Human Identification, Forensic Anthropology**

## W13    Recent Advances in Liquid Chromatography and Mass Spectrometry for Applications in a Variety of Disciplines in the Forensic Sciences.

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Upon completion of this workshop the attendee will: (1) be able to identify the most commonly used liquid chromatography and mass spectrometry techniques in the fields of forensic toxicology, seized drugs

and explosives, (2) gain an understanding of the theory and principles behind these techniques, (3) understand processes and variables that affect the quality of analytical results, and (4) learn about several specific forensic science applications of liquid chromatography coupled with mass spectrometry.

The fields of forensic toxicology and criminology encompass the analysis of therapeutic drugs, drugs of abuse, metals, and other toxicants in both biological and non-biological specimens. The scope of these disciplines are extremely broad and the reasons for testing include impairment investigations, urine drug testing, medication compliance monitoring, postmortem testing, and analysis of trace substances found at crime scenes. This presentation will impact the forensic science community by demonstrating newer types of instrumentation and the applications, including explosives that are now possible as a result of recent advances in the field.

The fields of forensic toxicology, criminology and explosives encompass the analysis of therapeutic drugs, drugs of abuse, metals, and other toxicants and incendiary materials in both biological and non-biological specimens. The scope of these disciplines are extremely broad and the reasons for testing include impairment investigations, urine drug testing, medication compliance monitoring, postmortem testing, analysis of trace substances found at crime scenes, and for homeland security. This workshop will appeal to a diverse audience with the common interest of learning about newer types of instrumentation and the applications that are now possible as a result of recent advances in the field. An introduction to commonly used liquid chromatography and mass spectrometry techniques in the forensic laboratory with a focus on recent technological advances useful in the analysis of illicit substances from a variety of sample matrices, including the analysis of seized drugs and explosives will be provided.

Forensic laboratories worldwide now recognize the impact liquid chromatography (LC) and mass spectrometry (MS) can have on their activities. The speed, selectivity and sensitivity of MS enables laboratories to confidently screen, confirm and quantify trace levels of drugs and toxins in a wide variety of biological, post-mortem and non-biological specimens. Over the past ten years rapid advancements in liquid chromatography, ionization techniques, and mass spectrometry have led to increased adoption and integration of these analytical techniques in forensic laboratories. The reduced requirements for, and in some cases obviation of, sample preparation prior to qualitative and quantitative analyses have hastened this trend while the increases in selectivity and sensitivity relative to other techniques, e.g., TLC, GC(/MS), and immunoassay, make this transition very attractive both scientifically and financially. In addition, modern analytical instrumentation including liquid chromatographs and mass spectrometers are more robust, easier to operate and occupy less laboratory space then their predecessors.

**Liquid Chromatography, Mass Spectrometry, Forensics**

## W14 Forensic Imaging: Current Developments and Future Directions

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The purpose of this workshop is to provide the participant with an overview of developments in forensic imaging taking place in Europe and the United States. After attending, the participant will be able to explain concepts of radiology assisted autopsy, list issues to consider in developing a program, and outline considerations required for incorporating new forensic imaging methodology into accepted forensic practice.

This presentation will impact the forensic science community by disseminating information on the evolving imaging techniques of high resolution CT and MR applied to autopsy practice.

The advent of high resolution multi-detector row CT scanners and fast MRI scanners in the last decade has allowed the development of imaging techniques that have greatly enhanced the diagnostic potential of these two imaging modalities. While conventional radiographs have played a valuable role in forensic diagnosis and practice for over a century, recent investigations with both CT and MRI suggest that these imaging tools are capable of much greater contributions. A major innovation is the ability to display imaging findings in 2D and 3D planes that closely replicate the findings at conventional autopsy and make the interpretation of the studies more easily understood by non-radiologists. CT and MRI may be used to supplement traditional autopsy techniques, to provide a complete anatomic assessment prior to limited autopsy, or in certain circumstances to replace it, such as in blunt accidental trauma, or drowning deaths. These studies may also provide options in the setting of religious and cultural objections to conventional autopsy.

While CT has the advantage of providing rapid whole body imaging of great anatomic detail in a short time, the superior contrast resolution of MR provides soft tissue characterization that is not achievable by CT. MRI is less widely available and more time consuming but may be applied to the postmortem evaluation of specific body parts to aid in the diagnosis of specific causes of death that may be characterized by subtle soft tissue changes. Both CT and MRI provide a permanent pictorial record of anatomic findings that may be retained and analyzed for medical and legal purposes postmortem and offer advantages in quality assurance that may be difficult to replicate with conventional autopsy.

The forensic science and medical examiner communities have shown interest in the use of CT and MR autopsy imaging. However, while CT and MR imaging are widely available in the clinical care of the living, forensic facilities face problems of access to autopsy imaging due to financial, technical, transportation, interpretation, and related difficulties. This workshop will address the issues of scanner purchase, maintenance and study interpretation costs, the potential for savings when conventional autopsy may be avoided, the need for wider research and validation of imaging autopsy techniques, requirements for training and certification of study readers, the development of protocols and

standards, and the acceptance of imaging findings by law enforcement and judicial authorities.

Addressing these issues is important if CT and MR imaging technologies are to become accepted by the forensic community at large and to be disseminated widely into forensic practice. Provided in this session will be an open discussion on the future integration of these advanced imaging techniques into death investigation and the autopsy process.

**Autopsy Imaging, Computed Tomography, Magnetic Resonance Imaging**

# W15 Deciphering the Code: How to Interpret Reports and Work with Forensic Scientists to Evaluate the Significance of Scientific Findings

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After attending this workshop attendees will be able to increase the efficiency of the flow of information between triers of fact and forensic scientists in courts of law. To this end, there are three objectives to this workshop: (1) to provide attorneys with methods for establishing a good rapport with expert witnesses prior to court so that information can be presented at trial in the most efficient manner possible, (2) to discuss and analyze report wording from numerous different sub-disciplines so that the information contained therein can be properly utilized in court, and (3) to discuss the relevance and significance of specific findings within those disciplines.

This workshop will impact the forensic science community by bridging a gap between triers of fact and forensic scientists. The net effect of which will be an increase in the flow of information between these two groups. By helping to make the flow of information between these groups more efficient, the legal process as a whole may become more efficient.

Although triers of fact and forensic scientists are tied together in the intimately woven fabric of the legal system, there are seemingly insurmountable obstacles that keep their worlds apart. The language of law and the language of science are, to put it succinctly, two completely different vernaculars. For the most part lawyers have minimal, if any, scientific training, while scientists rarely have any legal training further complicating communicative efforts in court. This breakdown in communication has some obvious implications within our legal system. The duet between an attorney and a scientist performing in a court of law can easily become somewhat discordant. If proper questions are not asked and the wrong questions are answered, it becomes very easy to move away from the presentation of relevant information and lose the interest of juries. The purpose of this workshop is to bring these two seemingly different groups together and provide a translational formula so that a harmonious result may follow.

As members of the scientific community at large, forensic scientists are bound by certain ethical standards. Ideally, the individuals practicing in the field should be unbiased in their interpretations of the evidence presented to them for analysis. They are not there to simply provide information to one side over another. To this end, most practitioners are more than willing to interact with and provide information to any interested parties be they affiliated with the defense or prosecution. Unfortunately, the system surrounding the scientists is adversarial in nature and there are often various obstacles preventing the free flow of information. Even when the information is readily available, the content is not always well understood. Navigating the jargon of the forensic sciences can be an arduous task. Often, relevant information can be buried deep within the notes of an analyst with only a short summary of relevant finding being listed on a report. The findings presented on such reports may also be somewhat vague and un-interpretable to the lay reader. Given such circumstances, how does one go about achieving effective communication with an expert witness?

It is no secret that proper communication is the key to a productive experience with an expert witness. In order to better communicate with their witnesses, ideally, attorneys should be familiar with them as individuals, have a good grasp on what they can and cannot say, and know enough about the science to be able to ask intelligent, probative questions. Such things, of course, are easier said than done. Therefore, the goal of this workshop is to provide attendees with a framework for establishing a good rapport with expert witnesses to better prepare them for interactions in court. Topics to be discussed will include how to establish good communication with an expert, the merits of pre-trial conferences, how attorneys can navigate the system to gain access to witnesses, how to assess the significance of scientific findings, and how to decipher reports. Attendees should leave this workshop with the sense that scientists and their results are easily approachable and the process involved does not necessarily need to be adversarial.

The faculty will consist of an attorney, a trace evidence expert, a fire investigation/fire debris analysis expert, a blood alcohol/toxicology expert, and a forensic biologist. Each individual will draw from their extensive experience to discuss the methods that they find are most effective for: (1) preparing for court, (2) presenting in court, (3) deciphering reports, and (4) assessing the significance of evidence in their particular area of expertise. After the presentations, the audience will have the opportunity to question the experts in a panel discussion type format so that any questions they might have can be answered from the various perspectives available.

**Scientific Reports, Expert Witnesses, Pre-Trial Preparation**

# W16 International Accreditation of Forensic Laboratories

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The goal of this workshop is to provide realistic expectations of what is required to achieve accreditation and demonstrate an achievable roadmap. The workshop will be geared to managers of international forensic laboratories with little or no exposure to the accreditation process.

This presentation will impact the forensic science community by assisting international forensic laboratories in creating a strategic plan to achieve ISO accreditation. The implementation of quality systems in countries currently without accreditation will improve the quality of work products and provide greater international acceptance of results. This is especially important in countries dealing with transnational

crimes and other issues of international interest such as human rights investigations.

With globalization, an increasing focus is being placed on the investigation of transnational crimes. Multi-country investigations and prosecutions require that the criminal justice systems in one country can use and trust information generated in other countries. Often evidence generated in forensic laboratories is at the center of multi-country investigations involving drug trafficking, cyber-crime, identity theft, corruption, terrorism, human trafficking and other transnational crimes. One important way to facilitate greater use and trust of information generated by forensic laboratories is through the adoption of common standards.

This workshop will provide an overview of the accreditation of multi-discipline forensic laboratory under ISO 17025. The workshop will be geared to managers of international forensic laboratories with little or no exposure to the accreditation process. The workshop will cover definitions of common quality assurance terms, the accreditation process, various accrediting bodies, the ILAC G-19 guidelines for forensic laboratories, facilities, and strategic planning and implementing timetables. The workshop will provide realistic expectations of what is required to achieve accreditation and demonstrate an achievable roadmap. The workshop will allow international forensic laboratories to create a strategic plan to achieve ISO accreditation.

**International, Accreditation, ISO**

## W17 Etiology of Serial Murders: Analyzing Behavioral and Psychological Perspectives

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After attending this workshop, attendees will have an understanding of the dynamics of serial murderers, their development, motivations, methods of operation, and their psychological characteristics. The goal of this workshop is to expose attendees to serial murderers, their development, motivations, methods of operation, and their psychological characteristics. The workshop will highlight historical and current trends in psychology pertaining to serial murderers, examine different motivations for serial murder, as well as the origins of those motives, and explore issues related to victim targeting and selection, as well as victim/offender interactions. Lecture will be supplemented by interview video clips of serial murderers discussing these issues and demonstrating identifiable traits.

This workshop will impact the forensic community by providing insight and understanding into the complex dynamics of serial murder and the etiology of serial murderers. This knowledge will assist the forensic community with identifying the unique characteristics of different types of serial murderers.

The FBI's National Center for the Analysis of Violent Crime (NCAVC) is routinely consulted by federal, state and local authorities in a variety of cases of bizarre and repetitive violent crimes, especially serial murder cases. The NCAVC is actively involved in research on serial murder. Dr. Louis Schlesinger is a nationally respected expert on serial murder and has written extensively about the dynamics of serial murder. This workshop will combine unique viewpoints to provide the attendees with an exclusive perspective regarding serial murderers and insight into the dynamics of serial murder.

Serial murder has long been an issue that receives considerable attention from academicians, medico-legal practitioners, and the media. Serial murder has been written about extensively and has been the subject of a great deal of research, much of which is focused upon the traits and developmental factors related to serial murder.

In the field of psychology, various theories have included serial murderers into an array of diagnostic categories, ranging from paranoid and narcissistic personality disorders to psychopathy. There have also been a number of serial murderers whose behavior has been attributed to organic factors, such as traumatic brain defect or injury. Causality has been discussed in terms of heredity, environment, and development.

The criminal justice community relies upon the information and insight provided by those professionals who have performed specialized research pertaining to serial murder to enhance their knowledge and effectiveness when investigating such crimes. This knowledge can enhance the efficiency and effectiveness in the investigation of an ongoing serial murder series, which in turn leads to an increased likelihood of successful apprehension and prosecution of the offender. To these ends, collaboration and communication between the various criminal justice entities and the medico-legal community ultimately reduces the public's risk of further victimization.

**Serial Murder, Serial Murder Dynamics, Causality**

## W18 Standards in the Forensic Sciences: Their History, Development, and Impact on Laboratory Practice

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After attending this presentation, attendees will have a better understanding of the standards that affect their everyday practice, and become aware of the interrelationships among the various standards development organizations.

This presentation will impact the forensic science community by raising awareness of the myriad groups tht promulgate standards for the benefit of the forensic science profession. Further, attendees will be encouraged to participate in the process.

The forensic science profession is the intended beneficiary of standards promulgated by no less than 10 national organizations. The purpose of this workshop is to bring together representatives of those organizations, with the goal of providing attendees with an integrated perspective about the standards each group promulgates, how they are promulgated, and how the standards directly impact laboratory practices.

Our profession has not always embraced standards, or even the concept of standardization. In days gone by, many forensic scientists held to the position that because each case was different, and because forensic science evidence samples tended to be small and non-homogeneous, it was not possible to develop "one-size-fits-all" protocols for handling evidence. Certain disciplines were able to overcome this resistance, and promulgate a few standard protocols.

However, prior to 1993 most laboratories had their own individual standards, which they, of course, believed to be valid, but no one had any way to know that for sure. Many testing methodologies were simply passed down by word-of-mouth. Then came *Daubert*.

The *Daubert* decision itself is a standard as evidenced by the opening paragraph in Justice Blackmun's opinion. "In this case we are called upon to determine the **standard** for admitting expert scientific testimony in a federal trial."

The opinion provided guidance to trial courts in federal cases, and the standard has been adopted by many states in the intervening 15 years. Among the issues that a judge *may* consider when ruling on the reliability of expert testimony is the existence of standards. In Justice Blackmun's words, "… in the case of a particular scientific technique, the court ordinarily should consider the known or potential rate of error, (citation omitted), and the existence and maintenance of standards controlling the technique's operation."

Standards instantly became a major focus of the forensic science profession, and because of a certain high profile trial in Los Angeles in 1995, forensic science standards became visible on the public's radar screen as well.

The modern paradigm for quality in the forensic sciences is represented by the forensic quality triangle, with the three legs representing standardization, accreditation, and certification. These three activities are all highly dependent on each other. Laboratories wishing to become accredited need to meet certain standards. Individuals wishing to become certified need to be knowledgeable about the standards that apply to their profession, and organizations promulgating standards need to be very cognizant of the use to which their work product will be put.

Many new standards begin their life as the product of a task group within a SWG or TWG. These groups can then subject their guidelines to a broader and more rigorous peer review provided by ASTM Committee E30 on Forensic Sciences, which is now one of the largest professional organizations in the forensic science industry. Standards promulgated by SWGDOC, SWGDRUG, TWGFEX, and SWGMAT covering everything from sample preparation to quality assurance to training have gone through this process.

Higher-level standards, dealing with ethics, laboratory management, and quality assurance are promulgated by ASCLD, and ASCLD-LAB. Standards for collecting and handling evidence in the field have been promulgated by NIJ. Most professional organizations in the forensic science profession promulgate individual standards of conduct in the form of ethical codes.

By the end of the day, attendees will have become acquainted with the history of standards development in the forensic science profession, the methods of standards development used by the various organizations that promulgate standards, and the interrelationships between these organizations that define our profession today.

**Standards, Accreditation, Admissibility**

# W19    Forensic Image and Video Processing

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After attending this workshop attendees will know the possibilities and limitations of state of the art video image processing techniques as well as 3D techniques.

This presentation will impact the forensic science community with the possibilities of image and video processing in court cases, quality assurance and new developments in this field.

During this workshop information will be provided on new developments of forensic investigation of (digital) images and video streams and the use of 3-dimensional computer modeling in forensic investigations.

Traditional sources of images as evidence concern crime scene photography, and more specifically, photographs of fingerprints, tool marks, shoe prints, and other impressions. A short overview of image processing techniques is given. Special attention is given to the introduction of artifacts by image processing (e.g., FFT on fingerprints), imaging in pathology and quality assurance aspects. During the last 35 years the use of CCTV-camera systems has become widespread. Typical questions concern the quality and the selection of images from a specific camera in a multi-camera-recording. Digital processing of video streams for presentation and storage purposes, and the compression techniques that are applied in digital CCTV-systems, lead to questions about the integrity and authenticity of recordings. Also questions about image interpretation like facial recognition, body length, or car speed, often in low resolution, time lapse, or compressed images have increased.

The use of image processing in the analysis of patterned injury of the skin, with emphasis on child abuse and as an aid in image analysis in forensic pathology will be discussed. The interpretation and recognition of image processing artifacts and image

New sources of video streams and images are video recordings from handy cams, digital photo camera's, internet, and cellular phones. Typical questions about these recordings concern the integrity and authenticity of the recordings, the data compression techniques used, the synchronicity of sound and images, compensation for camera movement, and the conversion of a video stream to a higher resolution image. This session will focus on methods for digital capture and analysis of analogue and digital multiplex surveillance recordings, state-of-the-art image enhancement techniques as contrast stretching and de-blurring, as well as methods as super resolution, stabilizing and automatic tracking.

Since more images are being processed for forensic investigation, new methods have been developed for answering questions about the interpretation of images. Examples given: is it possible to read a license plate number? is a suspect, or his car, the one depicted in the image? what is the body length of the robber or the speed of a car?, and, is it possible to do a reconstruction of an accident or a shooting incident from the information in these images? Methods for image comparison, facial

comparison with non standardized images, image reconstruction, and photogrammetry are presented and discussed. Special attention is given to accuracy of the results and the impact on the conclusions from these investigations. Furthermore, there will be hands on training during this workshop.

Finally, some extra attention is given to the use of 3-dimensional computer modeling in forensic investigations, since these techniques have an impact on traditional crime scene photography.

Computer models and animations have been recently used for analyzing video by superimposition of computer generated views of the model on the video images, for the visualization of complex scenarios in animations and for testing scenarios against video footage and evidence in crime scene photographs. Examples: the reconstruction of car accidents from photographs, analysis of blood spatter patterns from photographs using a computer model of the crime scene, the visualization of wound channels in computer models of human bodies, the reconstruction of bullet trajectories, the reconstruction of a burglary using the limited information in dark images from a multi-camera video recording, and the analysis of firework explosions from video recordings, photographs and geographical data. Special attention is given to modeling techniques, the accuracy of the models, methods for visualizing uncertainties and possibly erroneous suggestions coming from these visualizations.

**Image Processing, 3D Visualization, Detection of Manipulation**

# W20    ISO/IEC 17025:2005: Section 5.4.6 Estimation of Uncertainty - Is Anyone Certain What This Means?

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The goal of this workshop is to discuss the term "estimate of uncertainty" as required for accreditation under ISO 17025 and defined by the "ASCLD/LAB-International Estimating Uncertainty of Measurement Policy."

This session will impact the forensic science community by providing an opportunity to discuss the ISO 17025 "Estimation of Uncertainty" requirement.

The accreditation of forensic science laboratories is not a requirement; but it could be soon. The accreditation of the approximately 350 forensic science laboratories by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) will change from a two tiered program (Legacy and International) to exclusively the latter. The ASCLD/LAB International program will only accredit laboratories under ISO/IEC 17025:2005 requirements and ASCLD/LAB International Supplemental Requirements.

Laboratory accreditation under the International program will require compliance to approximately 300 requirements, most of which are fairly direct in terms understanding what must be done to conform. There is one ISO 17025 requirement which perhaps poses more questions than all other requirements combined:

5.4.6        **Estimation of uncertainty of measurement**

5.4.6.1     A calibration laboratory, or a testing laboratory performing its own calibrations, shall have and shall apply a procedure to estimate the uncertainty of measurement for all calibrations and types of calibrations.

5.4.6.2     Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement. In certain cases the nature of the test method may preclude rigorous, metrologically and statistically valid, calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data.

The degree of rigor needed in an estimation of uncertainty of measurement depends on factors such as: (1) the requirements of the test method, (2) the requirements of the customer, and/or (3) the existence of narrow limits on which decisions on conformity to a specification are based.

If the term "uncertainty" had a referenced, statistically valid definition which is usable, much of the uncertainty related to "uncertainty" would be minimized, if not completely disappear. Unfortunately, if ten different people are asked for a definition, there will usually be ten different responses. From these ten responses, there are questions related to another dilemma: How does the fairly intelligent, non-statistician plug numbers into a myriad of equations to answer the question "What does uncertainty mean?" Most laboratory managers approach this question from the perspective of "Tell me what to do and I will do it." The challenge then becomes one of obtaining a consensus opinion related to conformance to the requirements.

This workshop will provide a forum to discuss "Estimate of Uncertainty" from a perspective that a non-statistician can understand. The next challenge is to have information available which can be taken back to the laboratory for implementation. The ASCLD/LAB International *Uncertainty* document will form the basis of this discussion. There will be discussions of basic definitions from the literature to demonstrate this myriad of opinion.

**Estimation of Uncertainty, ASCLD/LAB International, ISO 17025: 2005**

# W21    Ethics and Forensic Science

*Tammy P. Northrup, JD\*, Office of Disciplinary Counsel, Louisiana Attorney Disciplinary Board, 4000 South Sherwood Forest Boulevard, Suite 607, Baton Rouge, LA 70816; and Paul Messner, JD, Federal Bureau Investigation, 2220 Grandview Drive, Suite 280, Fort Mitchell, KY 41017-1600*

After attending this workshop, attendees will have a thorough working knowledge of the background, history, and review of ethics in professions, the purpose of a Code of Ethics, the existing Code of Ethics for forensic scientists, and why ethics in forensic science are important. Attendees will be asked to consider whether a uniform Code of Ethics should be required for forensic scientists as it is required in other professions.

This workshop will impact the forensic community by initiating a dialogue to determine whether a uniform Code of Ethics should be required for all Forensic Scientists as it is required for other professionals.

A code of ethics outlines the principles of conduct governing an individual or group. Ethics in general address what is considered good or bad conduct and the corresponding obligations to a given situation. Many issues arising in professional situations involve complex questions that require an application of not only the law but also a system of values or principles. Codes of ethics provide professions with a degree of credibility. Inherent in a profession with a code of ethics is a regulatory system addressing complaints, investigations, hearings, and appeals.

Most professions mandate a code of ethics, but no such mandate exists for forensic scientist.

The fact that forensic science is a profession that has recently been cast in the limelight combined with certain recent action by some prosecutors and forensic scientists in several high profile cases have called into question whether an Ethics Code should be mandated for all forensic sciences. Said actions have also affected the admissibility of evidence in some criminal proceedings.

Research will be presented discussing the following:

- The history of ethics codes in forensic science;
- The current status of ethics codes in forensic science;
- The need for a uniform ethics code in forensic science;
- A discussion of recent court cases involving forensic science where the ethics of a forensic scientist or an attorney is at issue and the resolution of each case; and
- An interactive exercise where the participants in the workshop consider and discuss ten situations involving ethical issues in forensic science. Each of these situations is taken from actual events without disclosing any identifying information for the scientist, attorney, or jurisdiction involved. After the discussion and proposed solutions by the participants, the actual solution that was imposed in the case by the courts or appropriate governing authority will be revealed to the participants as well as how the actions of the forensic scientists or attorney affected the admissibility of any evidence in the case.

**Ethics, Admissibility, Regulation**

## W22 Pharmacology and Pharmacokinetics for Forensic Toxicologists

*David M. Benjamin, PhD\*, 77 Florence Street, Suite 107, North, Chestnut Hill, MA 02467-1918; and H. Chip Walls, BS\*, Forensic Toxicology Laboratory, University of Miami/Department of Pathology, 12500 SW 152nd Street, Building B, Miami, FL 33177*

The goals of this workshop are to: (1) summarize the basic physiology, pharmacology & pharmacokinetics required to interpret drug blood and urine levels which have been obtained both before and after death, (2) to present confounding issues that limit the interpretation of many quantitative blood test results, (3) to summarize drug interactions & drug-nutraceutical interactions that can affect drug toxicity, (4) to describe the pharmacokinetics of ethanol and related toxic alcohols: methanol, isopropanol, gamma-hydroxybutyrate, ethylene glycol and its precursor, 1,4-butanediol, (5) to identify factors involved in postmortem redistribution of drugs and their effects on the interpretation of postmortem drug test results, and (6) to examine the effects of pharmacogenomics and pharmacogenetics on drug toxicity.

This workshop will impact the forensic science community by reviewing the confounding issues in forensic toxicology and raise the awareness of the audience about the limitations post-mortem redistribution, genetic polymorphism, drug interactions and inter-subject variability place on the interpretation of toxicology test results.

Forensic toxicologists, pathologists, and criminalists are often presented with the results of a single blood test or a single urine test and asked, "Did it kill him/her?", "Was s/he impaired", or "Did it injure him/her? Such questions are very difficult to answer on the strength of one test result. Many times the investigating forensic scientist has to develop an adequate history regarding the time the last dose was taken, the amount, the route, and whether it was a single acute dose, large OD, or an OD that occurred due to drug accumulation over time. In addition, genetic factors regulating drug metabolism, drug interactions, and difference in drug disposition during toxic doses and therapeutic doses also affect drug toxicity.

When blood samples have been obtained after death or at autopsy, confounding changes in postmortem redistribution, putrefaction, bacterial contamination, and postmortem production of ethanol may require sampling from a variety of sites including vitreous and tissue samples from various organs to obtain enough data to address the issues of cause and manner of death. This workshop is focused on enhancing the forensic scientist's understanding of the pharmacology and pharmacokinetics involved with the interpretation of drug urine and blood test results.

A review of the physiology, pharmacology and pharmacokinetics of drugs, including presentations on drug interactions, genetic polymorphism, and postmortem redistribution will be presented followed by a panel discussion and presentation of selected cases. The toxicity of ethanol and other toxic alcohols and glycols will also be discussed, as well as the toxicity of GABA. Attendees will be encouraged to share information about interesting cases in which they have been involved.

**Postmortem Redistribution, Drug Interactions, Genetic Variability**

## W23 Operation Street Smart: An Overview of Current Street Drugs and Drug Culture

*John F. Wyman, PhD, and John R. Sudimack, BS, Franklin County Coroner's Office, 520 King Avenue, Columbus, OH 43201; and Mike N. Powell\*, Steve Tucker\*, and Shawn Bain\*, Franklin County Sheriff's Office, Special Investigations Unit, 410 South High Street, Columbus, OH 43215*

After attending this presentation, participants will gain up-to-date and in depth knowledge of what street drugs are, the current drug culture and the trends for the future. They will be able to recognize, what was probably previously unnoticed, drug related behavior, terminology, paraphernalia, dress, and physiological signs of drug use.

The United States demands and abuses more drugs than any other country. This demand has turned drug trafficking into a competitive business that generates billions of dollars for major drug cartels throughout the world. We are engaged in a Drug War, the outcome of which is in doubt, without the positive involvement of families and communities. Most of us are parents or will be parents. This workshop will impact the forensic science community by empowering us to build a foundation for drug abuse education for ourselves, families and our communities. This presentation will raise the consciousness of the audience about the global size of the "Drug Problem" (it is bigger than you think), and educate them about the signs and symptoms of drug abuse in our young people (possibly our own children).

Operation Street Smart was created in July 2002 as a collaborative effort to provide current information on trends, terminology, paraphernalia, and physiological effects of illicit drugs. This endeavor is the first of its kind in the United States and last year received the FBI Director's Community Leadership Award. The workshop includes actual examples of current designer street drugs such as XTC, AMT, 5-Meo-DMT, LSD, GHB, ketamine, and khat. D.A.R.E. officers escort the examples throughout the audience for hands on effect. Current drug paraphernalia examples are available to depict the ease in camouflaging drug use from adults. A strong emphasis is placed on the physiological effects of the drugs and indicators to look for. So-called "traditional" drugs such as marijuana, cocaine, crack, heroin, and methamphetamine are also covered extensively as teenagers still heavily abuse these drugs. A portion of the program also deals with prescription medications, including DXM, because of their easy accessibility in most households.

**Illicit, Drug, Abuse**

## W24 Advances in Archeological Approaches to Crime Scene Investigation

*Ian Hanson, MSc\*, and Paul Cheetham, MSc\*, Bournemouth University, Room C136, Christchurch House, Talbott Campus, Fern Barrow, Poole, Dorset BH12 5BB, UNITED KINGDOM; and Steven V. Gilbert, PhD, 287 Cowan Road, Canton, NY 13617*

The goals of this workshop are to: (1) provide knowledge and technical detail of archeological applications to CSI, (2) to provide case studies of how techniques and methods are employed, (3) to provide details of technical equipment being employed, and (4) to increase understanding of management approaches to archeological CSI approaches.

This presentation will impact the forensic science community by demonstrating advances in the use of archeological techniques and methods for CSI, and describing how the development of technical equipment used will provide crime scene investigators and forensic scientists with the latest cases, applications, research and management approaches.

Why involve archeologists in forensics? This workshop discusses practical reasons why. Archeologists have increasingly been used for excavation and recovery in crime scene and other forensic investigations as they demonstrate they have expertise in untangling the sometime chaotic structure of scattered and buried features, artifacts and deposits.

This session provides an overview and description of developing and new approaches to CSI from the archeological perspective. In the last five years, new technical approaches and refinement of methods based in archeological sciences have been used in forensic investigations, and developments have been research, and assessed for their potential application to investigations.

These new approaches and how traditional archeological methods have been selected as appropriate for forensic use will be discussed, and illustrated with case studies.

How archeology is now used across a breadth of investigations and how archeologist can collaborate with multi-disciplinary teams investigating crime scenes will be discussed; archeologists have a key role on some crime scenes in evidence location, identification, and recovery. Management of archeologists and archeological methods at scene are discussed.

**Forensic Archeology, CSI, Advances**

## A1    The Future of Forensic Science: Reflection of the Last Thirty Years of Criminalistics

*Thomas A. Brettell, PhD\*, Cedar Crest College, Department of Chemical & Physical Sciences, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will better understand the accomplishments in the field of Criminalistics over the last thirty years and what new technologies that may impact the field in the future.

Forensic science has made great strides within the last few decades. No one would argue that great accomplishments have been made in every discipline within the field, but the technological innovations which had the greatest impact may have been in criminalistics. Twenty years ago crime laboratories were not performing DNA analysis. Today, DNA analysis is a highly effective routine tool for forensic scientists. The impact of STR technology along with the use of databases has transformed crime laboratories from service organizations to investigational agencies with a much more effective crime-fighting role. The development of capillary electrophoresis (CE) theory and instrumentation in this period has also played an important role for DNA analysis becoming a rapid, sensitive, and routine tool in the forensic laboratory. In addition, the development of mitochondrial DNA has led to missing persons databases that have become effective in identifying unidentified remains.

While DNA technology was grabbing almost everyone's attention perhaps the most overlooked technological development in criminalistics is the development of the union of microscopy and spectroscopy. Over the past several decades the ability to perform spectrophotometry and spectroscopy on very small samples has dramatically improved. The improvement of microspectrophotometry and a host of other similar instrumentation has provided trace evidence examiners an arsenal never envisioned by Edmond Locard. The technological developments in the laser field have brought the technique of raman spectrophotometry onto the list of tools for the criminalists. The ability of raman spectrophotometry to analyze small samples and also analyze materials such as drugs directly through the packaging without disturbing the actual sample has had a significant impact in both trace evidence and drug analysis. In addition, the development of portable hand-held instruments is bringing more analytical chemistry to the crime scene than ever before. The ability to immediately preliminarily identify unknown substances, such as anthrax and explosives provides crime scene investigators with information that took days and weeks in previous years.

The maturing of hyphenated techniques has also significantly impacted the forensic science field. In particular, the availability of affordable table-top gas chromatograph/mass spectrometers has given the majority of crime laboratories world-wide the ability to reduce or even eliminate the drug and toxicological analysis backlogs that many faced in the 1980's and early 1990's. Gas chromatography/mass spectrometry (GC/MS) has replaced gas chromatography-flame ionization detection (GC-FID) for the analysis of fire-debris samples. Other hyphenated techniques such as liquid chromatography/mass spectrometry (LC/MS), gas chromatography/infrared spectrophotometry (GC/IR), and tandem mass spectrometry techniques (LC/MS/MS and GC/MS/MS) once only found in academic research laboratories have now become routine instrumentation in the modern well-equipped drug and toxicology laboratories. Other hyphenated techniques such as inductively-coupled plasma/mass spectrometry (ICP/MS) are also impacting the crime laboratory because of the affordability and simplification of operation.

Forensic applications using techniques such as matrix-assisted laser desorption ionization (MALDI), surface-excitation raman spectroscopy (SERS), isotope ratio mass spectrometry (IRMS), terahertz reflection spectroscopy, ion-mobility spectrometry, DART Mass spectrometry, CE/MS and CE/MS/MS are already appearing in the literature. These techniques and other technology such as nanotechnology, and miniaturization such as chip technology, will have a major impact and reshape the analytical chemistry in forensic laboratories in the coming years. The future looks bright and exciting in the field of Criminalistics!

**Forensic Science, Criminalistics, Analytical Chemistry**

## A2    The Future of Forensic Science Education

*Max M. Houck, MA\*, 1600 University Avenue, 208 Oglebay Hall, West Virginia University, Morgantown, WV 26506-6217*

After attending this presentation, attendees will better understand the status of forensic science education efforts, including programs, graduate programs, accreditation, and curricula, with an international perspective.

This presentation will impact the forensic community by providing a perspective for forensic science education to grow and improve as the foundation for forensic science as a separate profession.

Forensic science education has become a global enterprise and the growth of programs worldwide has spurred various efforts towards improving their quality and validity. Accreditation programs in the United States and the United Kingdom have been instituted in the last few years as a check on the rapid growth of programs in response to the popular media. A set of controlled relationships exists in Australia which guide and shape academia's response to the profession's needs. Canadian, with more programs in Australia, faces a choice between the United States, the United Kingdom, and the Australian models. Concurrently, the profession has begun to embrace accredited programs and has recognized the value of such a process; however, some still do not see the value of a separate forensic science degree. The accreditation programs, for their part, have been seeking external recognition for their processes to legitimize their efforts to the community. All of this is occurring in an environment where forensic science is under siege, accused of a variety of sins, including negligence, incompetence, and cronyism.

The stage is set for forensic science to step into its next phase of development. Any profession is only as good as the educators who incubate its practitioners. For forensic science to improve, forensic science educational programs must be the first bastion of quality. They cannot do this, however, without a solid, complicit integration with the profession and its stakeholders. Therefore, forensic science faces a choice: Continue on a path to being "mere technicians" at the mercy of attorneys and the courts or to strap on the responsibility of being a true and separate science.

**Education, Accreditation, FEPAC**

## A3    Where is the Science?

*Peter R. De Forest, DCrim\*, John Jay College/CUNY, 445 West 59th Street, New York, NY 10019*

After attending this presentation, attendees will have discussed the balance between science and technology in criminalistics laboratories.

This presentation will impact the forensic community by stimulating discussion and drawing attention to the need for assuring that the work in criminalistics is science based.

This paper will pose several questions related to an apparent trend toward the replacement of science by technology and scientists by technicians in modern criminalistics laboratories.

As more advanced off-the-shelf technologies and more sophisticated automated analytical instrumentation become available in forensic science laboratories, questions to be answered will be: are criminalists doing less science? Is the use of standardized thresholds or cut-offs and uniform reporting criteria always better than interpretations that take case specifics into account? Does uniformity of reporting designed to minimize false inclusions run the risk of missing exclusions? Should there be symmetry with respect to the criteria for reporting of both inclusion and exclusion criteria? When proprietary off-the-shelf technology is employed in the form of pre-prepared kits, does/can the user understand the mechanism well enough to recognize and guard against unanticipated interferences and possible interpretation errors? When computer-controlled and highly sophisticated instrumentation is it being operated, is it employed in the manner of a "black box" device with a technician approach, or is the theory behind its operation understood so that it can be employed as a scientific tool in dealing with special problems? Is the instrumentation selected the best for the task at hand, or is it selected because it generates impressive data? Can anything but routine, predefined problems be addressed by what are primarily technological solutions? Is science available to address complex problems when they are encountered and recognized?

**Criminalistics, Science, Technology**

## A4    The Future of Fire and Explosion Investigations and Analyses

*Carl E. Chasteen, BS\*, State Fire Marshal, 38 Academy Drive, Havana, FL 32333*

After attending this presentation, attendess will gain information on a recent survey as to the future needs and trends in fire and explosion analyses and investigations.

This presentation will impact the forensic community by presenting the findings of a national survey as to the needs and trends in fire and explosion investigations and analyses. It will include a discussion of training as well as development of instrumetation and investigative tools. This will provide direction to analysts, students, researchers, and course developers.

Among the various types of criminal investigations and the varied specialties for forensic analyses, crimes associated with arson and explosions are sometimes the most difficult to process and analyze. The inherent destructiveness of the events often compromise much of the evidence left behind. Ignitable liquids and many individual chemical compounds are found as contaminants in various matrices from a fire scene. The residues produced from the complete reaction of explosives are often gases. Those, which are not gases, are often so common that their presence is not meaningful.

While various professional organizations of forensic scientists and investigators have a high level of interest and desire in improving both the procedures at the scene and the capabilities of the laboratory, the status of investigations and analyses are not uniform across the nation. Among many scene investigators, there is a desire to use more scientific and forensically sound methods. Among laboratory analysts, there is a desire to be able to glean the most that science can reveal about the evidence and to attempt to approach the same levels of individualization as has been achieved in DNA analysis.

Recognizing the current state of affairs and wishing to provide guidance, the National Institute of Justice through the National Center for Forensic Science worked to assess the near- and long-term needs in Arson and Explosion analyses and Investigations. This work was primarily completed through a select committee of Technical Working Group for Fire and Explosions (TWGFEX) members. The assessment included TWGFEX members as well as additional representatives of the relevant communities. A survey instrument targeted to those communities was prepared. The survey results were collected in late September of 2007. The TWGFEX panel met in late September of 2007 to discus the results of the survey. The group drafted their recommendations for a report which has been submitted to the National Institute of Justice. This presentation will provide an overview of that report and the results from the survey instrument.

**Fire, Explosion, Arson**

## A5    Forensic DNA:  Perspectives on Progress in a Rapidly Growing Field

*John M. Butler, PhD\*, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD 20899-8311*

The goal of this presentation is to describe recent developments and the state of forensic DNA analysis with comment on the potential and future of this rapidly growing field.

This presentation will impact the forensic community by explaining where the field of forensic DNA testing has come and where it is going. Challenges in terms of education and growth will be addressed.

Since its introduction in the mid-1980s, forensic DNA testing has played an important role in the criminal justice community through aiding conviction of the guilty and exoneration of the innocent. Remains from missing persons and victims of mass disasters have been re-associated and identified through linking reference samples to recovered remains. New technologies are regularly introduced and validated to expand the capabilities of laboratories working to recover DNA results with improved sensitivity and informativeness.

As of July 2008, the U.S. national DNA database contains over six million profiles and has aided tens of thousands of investigations nationwide. The success of DNA has resulted in an expansion of DNA collection laws from offenders and arrestees and a dramatic increase in the numbers of samples submitted for analysis from crime scenes. Forensic laboratories have embraced automation for sample preparation and data interpretation in order to meet increasing throughput demands. Short tandem repeat (STR) typing continues to be the primary workhorse in forensic DNA analysis although new genetic markers are under development for specific applications.

The general public continues to be interested in forensic DNA in large measure due to the popularity of TV programs such as *CSI: Crime Scene Investigation* and *Law & Order*. Other fields such as genetic genealogy and biometrics are increasing interfacing with forensic DNA methodology. Some of the biggest challenges facing the field today are education and training of new staff so that growth in the area of DNA testing can be addressed.

**Forensic DNA, STR Typing, DNA**

## A6 Forensic Thinking or Thinking Forensic?

*Eric Stauffer, MS\*, School of Criminal Sciences - University of Lausanne, Batochime, Lausanne, 0 CH-1015, SWITZERLAND*

After attending this presentation, attendees will have been provided with fodder for discussion regarding the future of forensic sciences from a general perspective and will ask attendees to personally reflect on whether or not they are making good decisions for the field and are leading the forensic community in the right direction. The attendees will clearly understand how forensic scientists have been positioned historically, in the present day, and in which future directions they may go.

This presentation will impact the forensic community by providing key elements for a complete reflection on where the forensic sciences stand today. This is a great starting point for those who represent the future of the profession and for those who will shape the next generation of forensic scientists. Many changes and evolutions are taking place in the forensic community, and now is the time to make adjustments in order to ensure that forensic scientists are heading in the right direction.

The foundation of modern-day forensic sciences is now more than 100-years-old. The great work of pioneers Hans Gross and Edmond Locard set the stage for forensic scientists today. Forensic sciences remain here today to assist the court in discovering the truth in both criminal and civil litigations. In the 1960s, tremendous technical improvements allowed forensic scientists to further advance the field. When used in perfect conjunction with the proper thinking process, these new technologies led to a betterment of the field. In the last decade, technologies have also undergone tremendous developments. Back in the early days, a test tube was about the most advanced piece of equipment found in a crime laboratory, but today, it is not unusual to find a gas chromatograph-mass spectrometer or some other gizmo used to measure isotope ratios. Unfortunately, these recent technological advances, while constituting great technical improvements, have not only not led to an improvement of forensic sciences, but have also contributed to its decline.

New technological advances have three major consequences for the forensic community to consider. First, because the amount of data obtained from an analysis is so large and because the analytes detected are so minute, forensic scientists often lack the capacity to understand the significance of the results. Second, because the analyses are so complicated, forensic scientists now are more focused on understanding these analyses rather than understanding the evidence, itself, and what it means in the particular context of an investigation. In addition, the level of specialization is so high that modern forensic scientists no longer have a global view on a case. Third, because the instrumentation now is so complex, the recent trend is to form technicians who can operate an apparatus that spits out results, rather than scientists who can actually read the results and interpret what they mean.

It is crucial that every forensic scientist fully comprehends the genuine forensic thinking process and constantly applies it in his/her

duties. In the early days of forensic sciences, the fathers of criminalistics made it clear that forensic sciences revolved around the evidence. Nowadays, when one looks at how recent research is conducted and how some academic programs are designed, it too often appears that the philosophy is that forensic sciences revolve around the techniques used to analyze the evidence and no longer the evidence, itself. It seems blatantly obvious that forensic sciences have slowly deviated from scientific rigors toward legal and political concerns. However, what the forensic community must remember is that forensic scientists first serve science and then legal concerns. It is not acceptable to deviate from science to satisfy legal concerns. It is not acceptable to ignore scientific certainties, if any, to become more politically correct. It is not acceptable to ignore circumstances and botch the interpretation of evidence.

Forensic scientists applying proper forensic thinking are an endangered species. It is now time to reverse the stream and to spread the genuine forensic thinking process again in order to ensure a bright future for forensic sciences and for the fulfillment of their mission to justice.

**Forensic Sciences, Future, Thinking Process**

## A7 Mystery Solved: The Identification of the Two Missing Romanov Children by Forensic DNA Testing

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After attending this presentation, attendees will understand forensic DNA testing that was conducted on a set of skeletal remains recovered in the summer of 2007 near the former mass grave of members of the Romanov family officially excavated in 1991.

This presentation will impact the forensic community by concluding one of the greatest mysteries of the 20th century.

For over 300 years, the Romanov Dynasty ruled the country of Russia. In 1917 following the Bolshevik revolution, the last Russian Tsar, Nicholas II, abdicated his throne and was eventually exiled to Yekaterinburg with his wife, Tsarina Alexandra, and their five children: Olga, Tatiana, Maria, Anastasia, and the Tsarevich Alexei. Also present with the royal family were four loyal servants: Dr. Botkin, the family physician; Mr. Trupp, valet to the Tsar; Ms. Demidova, maid to the Tsarina; and Mr. Kharitonov, the family cook.

In July of 1918, the Bolsheviks feared an attempt to rescue the Tsar and his family by the White Russian Army. A decision was made by the Bolsheviks to execute the entire family, with the hope that upon hearing of the Tsar's death the will of the people loyal to the Tsar would be broken. In the early morning hours of July 17, 1918 the royal family and their servants were led to the basement of the Ipatiev house where they were being held and were executed.

According to the account written by the lead executioner, Yakov Yurovsky, the Bolsheviks first sought to dispose of the bodies by throwing the remains down an abandoned mine about 20 km outside of

Yekaterinburg, and then attempting to collapse of the mine by exploding grenades down the shaft. This strategy did not work as planned, and the next night the Bolsheviks endeavored to move the remains to another mine shaft about 30 km away. Approximately 2 km from the original mine shaft, their truck broke down in an area known as "pig's meadow." According to reports, the Bolsheviks removed two of the children's bodies from the truck at this location. In an attempt to completely destroy the remains, they dug a shallow grave, doused the bodies in sulfuric acid, and burned them as much as possible. This effort took more time than anticipated, however, and the remaining bodies were doused with sulfuric acid and hastily buried together, "some distance away", in a mass grave.

Approximately five days later, Yekaterinburg was liberated by the White Russian Army, and an attempt by investigators to discover the remains of the Romanov family came to a dead end. In the late 1970s, two Russian citizens obtained a copy of the Yurovsky report and were able to locate the mass grave containing the remains of five members of the royal family and their four servants. Following the fall of the Soviet Union in 1991, the men came forward with their discovery and an official recovery was conducted.

Forensic DNA testing of the remains recovered in 1991 was conducted by Dr. Peter Gill, formerly of the Forensic Science Service and Dr. Pavel Ivanov, a Russian geneticist.[1] Nuclear STR testing of five loci confirmed the sex of the skeletons and established a familial relationship among the remains of the Tsar, the Tsarina and three of their daughters recovered from the same grave. Mitochondrial DNA testing confirmed a maternal relationship between HRH Prince Philip, the Duke of Edinburgh, and the Tsarina (and her daughters). The Duke of Fife and Princess Xenia were used to match the putative remains of the Tsar. A single point heteroplasmy at position 16169 (C/T) was observed in the mtDNA sequence of the Tsar, whereas his maternal relatives were fixed for 16169 T. In testing conducted at the Armed Forces DNA Identification Laboratory (AFDIL), the identity of the Tsar was further confirmed by the mtDNA heteroplasmy shared with Grand Duke Georgij Romanov, brother of Tsar Nicholas II.[2]

Despite the overwhelming forensic evidence, doubts about the authenticity of the remains persisted. Skeptics often referenced the two children missing from the mass grave - Alexei and one of his sisters, either Anastasia or Maria. The missing remains also encouraged the beliefs held by some that these two children had somehow miraculously escaped the bullets of the executioners and made their way out of Russia. The most famous case was Anna Anderson, who claimed to be the Grand Duchess Anastasia. Forensic DNA testing eventually disproved her claims.[3]

After the discovery of the "first" mass grave, several attempts were made in the following years to find the "second" grave, which was believed to be relatively nearby. In the summer of 2007, a group of amateur archeologists discovered a set of 44 bone fragments and teeth approximately 70 meters from the "first" grave. In late 2007, the Russian government invited a team of scientists to conduct an independent forensic DNA testing of the remains from the "second" grave.

We will present results from mtDNA, nuclear STR, and Y-STR testing of these remains. DNA testing on the skeletal remains from the "first" grave that conclusively link the two graves will also be presented. The results from the DNA analysis of all three genetic systems confirms that the samples tested from the second grave are one female and one male child of Tsar Nicholas II and Tsarina Alexandra, and resolves the mystery of the missing Romanov children.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, or the U.S. Department of Defense.

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**Romanov, DNA Testing, Human Identification**

## A8    We're Not Gonna Take It!  The Sequel (Case Studies From New York City)

*Noelle J. Umback, PhD\*, Office of the Chief Medical Examiner Department of Forensic Biology, 421 East 26 Street, New York, NY 10016*

After attending this presentation, attendees will have viewed several case studies. In each, DNA from the assailant was obtained after the victim, in the course of fighting back against her assailant, collected biological evidence useful for DNA testing. To date, CODIS has solved some of these cases, although others remain unsolved.

This presentation will impact the forensic community by demonstrating that semen need not be the only target for DNA analysis in rape cases, nor blood in homicides. Women who defend themselves during an attack help forensic laboratories by safeguarding or creating evidence that might otherwise never exist at all or be lost.

The original presentation of this topic presented at the New Orleans meeting of AAFS (2005) was very well-received and proved to be entertaining as well as informative.

The combination of quick-thinking by victims (including one who was killed consequent to fighting back), thoughtful analysis of the evidence and its application to the case itself resulted in the discovery of critical case evidence which in turn aided each criminal investigation.

The following are a samples of the cases which will be presented:

A 68-year-old homeless woman in Manhattan managed to pull a gold chain off the neck of a man who sexually assaulted her in a park in 2005. A swabbing of the necklace yielded a mixture of DNA, from which her own DNA could be subtracted to give the male's DNA - which was also consistent with semen evidence in the case. Once uploaded to CODIS, the male profile matched DNA collected from the fingernail scrapings from a victim in an unsolved attempted sexual assault committed in Miami-Dade County (part of a pattern of three from that jurisdiction). Two years later, in 2007, New York State entered the profile of a man convicted of robbery into SDIS and found a match to all four cases. He is scheduled to go on trial for the 2005 Manhattan sexual assault in summer 2008.

A lesson in don't attempt to steal what is most important to a woman – her handbag! A Brooklyn woman fought back when a stranger snatched

her purse – scratching him with such force that the blood from her hand was later swabbed at the police station. Her attacker was in such a hurry to run off that he also left behind one of his shoes. The profile from the blood matched the DNA from the shoe. Once uploaded into New York's SDIS, the profile matched a parolee who has spent the better part of the past 25 years in prison - for multiple convictions - including rape, sodomy, and robbery. His parole has now been revoked.

A shocking ending to a crime which begin with blaming the victim for possibly exposing herself to the unsavory world of the internet. An 18-year-old girl was found murdered in her ransacked home in Staten Island in 2005. Though the crime scene showed obvious signs of her attempts to fight off her assailant, she had ultimately been smothered to death. Police immediately focused in on this young woman's extensive chat-room activity on the internet and the numerous men she had consequently met. While the police first believed her own social choices had contributed to her death, examination of the fingernail scraping collected postmortem revealed biological material under all ten of her fingernails. The full DNA profile of her killer was developed. Her half-brother was convicted of her murder and sentenced to 18 years—one for each year of her life.

A woman in Queens was dragged off the street and into a darkened area, strangled and raped by an unknown assailant. However, prior to being choked into unconsciousness, she managed to swipe or scratch her fingers in his nose. Biological material collected from under her fingernails yielded a clean 12-locus male profile. This was uploaded into the CODIS system and almost three years later, matched a New York State offender who had been compelled to provide a DNA sample as a consequence of his conviction for misdemeanor petit larceny. He would later tell police "the voices in his head told him to do this." Evidently the voices haven't persuaded him to plead guilty; he is awaiting trial.

**CODIS, Sexual Assault, Self-Defense**

## A9 Hyperspectral Imaging Provides Easy Detection and Visualization of Biological Stains

*Paul E. Kish, MS\*, Forensic Consultant & Associates, PO Box 814, Corning, NY 14830; and Rebecca L. Schuler, BS\*, ChemImage Corporation, 7301 Penn Avenue, Pittsburgh, PA 15208*

After attending this presentation, attendees will understand the basic principals of hyperspectral imaging and how it compares to conventional methods of detection, visualization, and examination of biological fluid evidence on various types of substrates.

This presentation will impact the forensic community by introducing a new method for capturing and enhancing images of biological fluid evidence.

At most crime scenes, biological evidence is present in various forms. Human biological fluids, blood, semen, and saliva are of particular interest to the forensic examiner. The ability to utilize these fluids to make critical forensic links between the victim(s), accused, items of evidence, and the crime scene are unquestionably significant. The significance of biological fluids can never be realized without first being able to locate them on substrates in a non-destructive manner. With bloodstain patterns we not only need to locate the stains but there is a need to be able to visualize the physical characteristics of the stain patterns to assess their entire forensic value. The physical characteristics of the bloodstain patterns can provide the examiner with information as to how the bloodstains were deposited onto the substrates. This

information can assist with establishing whether a crime was committed, what type of crime was committed, where the crime was committed and who may have committed that crime.

The locating of biological fluid evidence can be challenging. Conventionally, searching for and capturing biological fluid evidence is performed by visually scanning the evidence with light sources such as, high intensity lights and an alternate light source (ALS). Often, different types of excitation wavelengths and colored goggles or barrier filter combinations are attempted in order to maximize contrast between the biological fluid and the substrate. Searching for bloodstains on dark, patterned or otherwise interfering substrates is especially difficult. These substrates unquestionably inhibit our ability to assess the physical characteristics of bloodstain patterns.

In this study, two methods of imaging biological fluid samples were evaluated based on the technologies' ability to detect, discriminate, and categorize the samples, as well as provide images with strong sample-to-substrate contrast. The first method of examination is visual inspection and digital photography. This method involves using various excitation wavelengths and barrier filter combinations, chosen based on educated information regarding the emission, absorbance, or reflectance properties of the fluid of interest. The second method of biological fluid sample examination is hyperspectral imaging. Hyperspectral imaging combines digital imaging technology with conventional spectroscopy for evidence analysis. It provides high spatial resolution, high image definition, and full spectrum analysis. In operation, digital images of the sample are recorded as a function of wavelength through the use of an electro-optic imaging spectrometer, generating a fully resolved spectrum for each pixel location in the multi-frame image. The combined spatial and spectral information reveals subtle features of a material that, often, cannot be observed using traditional imaging techniques.

Pure biological samples (blood, saliva, and semen) were deposited onto various substrates, including dark colored cloth and plastic, light colored cloth and plastic, and patterned cloth. The samples were examined using both technologies. The results demonstrate the strengths and weaknesses of each methodology, including the ability of each to produce images with maximum sample to substrate contrast and stain pattern visualization.

**Hyperspectral Imaging, Bloodstains, Digital Photography**

## A10 Forensic Discrimination of Blood on Various Substrates by Diffuse Reflectance Infrared Spectroscopy (DRIFTS) and Visualization Using a Sensitized Thermal Detector

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The goal of thi presentation is to present studies establishing a scientific basis for the spectroscopic detection and discrimination of blood stains from other background materials that might be present at a crime scene. Attendees will learn about the development of a prototype camera for rapid and nondestructive visualization of blood at crime scenes.

This presentation will impact the forensic community by explaining the development and design of a novel detector for visualizing blood stains at crime scenes.

Developing techniques for the visualization of biological fluid stains

on common surfaces has been a continuing goal for numerous forensic studies. The ideal device would be small, relatively inexpensive, and easy to operate and maintain portable; enhancement reagents would not be necessary; the method would be nondestructive; further, the device would detect trace levels of blood; and operate indoors or outdoors under ambient lighting.

A prototype imaging device is being developed that combines selective filters with a modified thermal array detector having a spectral response sensitized for blood detection. This goal will be achieved by modifying the detector with a metal mirror followed by the polymer film so that the film absorbances are responsible for most thermal conversion. By using polymers that mimic the spectral signatures of biological fluids of interest (e.g., blood, semen, saliva, and urine), the location of deposits of these fluids can be detected even in the presence of potential interferents that might be expected at crime scenes. The scientific basis and design characteristics of such a detector in systematic experiments using diffuse reflectance infrared spectroscopy (DRIFTS) have been developed. IR spectra of blood proteins such as hemoglobin and albumin exhibit distinctive IR absorbance due to the amide I and amide II bands in the 1650-1540 wavenumber range. In preliminary experiments, multiple substrates (textile and carpet polymers such as nylon, acrylic, cotton, oflefin, and polyester) have been tested, before and after doping with various concentrations of blood.

Multivariate statistical analyses were employed to determine the optimal spectral region for discrimination between neat and blood-doped substrates, and to measure false positive/negative error rates. Classification accuracies ranged between 96-100% comparing neat and bloodstained substrates, with little to no false negative misclassifications.

With no alterations to a Merlin un-cooled microbolometer camera system, differences between infrared images taken with and without blood stains were seen as well as being able to distinguish the stains. Both experimental results and simulations will also be shown to validate the design parameters of our imaging instrument. Ongoing research on combinatorial optimization of detector design parameters will be discussed, along with practical evaluation tests.

**Blood Stains, Diffuse Reflectance Infrared Spectroscopy, Thermal Detector**


## A11    Blood and Tissue Spatter Associated With Chainsaw Dismemberment

*Jessica E. Lichty, MA\*, Sioux Falls Police Department Crime Lab, 320 West 4th Street, Sioux Falls, SD 57104; and Brad Randall, MD, 2441 Stanton Drive, Sioux Falls, SD 57103*

After attending this presentation, attendees will recognize and understand blood and tissue spatter patterns characteristic of postmortem dismemberment of a human body with a small powered electric chainsaw.

This presentation will impact the forensic community by serving as an evidentiary resource for investigating the potential dismemberment by chainsaw of a human body, when not immediately evident from scene investigation.

In February of 2005, a 43-year-old, 108 kg, woman was reported missing. The ensuing police investigation and evidence found at the suspected scene suggested that the missing woman had been killed and subsequently dismembered in the basement of the residence. A part of the incriminating evidence was the recovery of a receipt for an electric chainsaw, which was never recovered. The location of the suspected dismemberment was in the basement of the home in a small confined space once used as a coal room (approximately 2 x 3 x 3 meters) with a concrete floor.

Several weeks into the investigation, the entirety of the victim's remains had been located, to include two lower legs amputated below the knees, an intact pelvic region amputated below the navel, and an intact torso. The pelvis and legs recovered from a local landfill, while the torso was found in a neighboring state. Those investigating the crime; however, were uncertain that an electric chainsaw could have been used to dismember a large human body in such a small, enclosed, space with such little evidence of blood or tissue spatter. There also was skepticism that the small electric chainsaw apparently purchased by the assailant could be powerful enough to dismember a large body without becoming fouled in soft tissue and bone.

To address the above questions two experimental reenactments of the dismemberment were conducted. The same make and model of electric chainsaw, as noted on the recovered receipt at the scene, was used in the experiments. White cotton sheeting was used to recreate the dimensions of the small basement coal room. Two humanely euthanized female pigs were used as the test carcasses. The experiments showed that an electric chainsaw easily cut through the pig carcasses with little resistance beyond some mild to moderate pressure needed for the initial skin penetration. In the first experiment, with the saw held largely parallel to the floor, there was a trail of tissue deposited largely directly beneath the chainsaw bar and a somewhat larger puddle of tissue on the floor directly under the discharge chute of the chainsaw. Very little spatter, consisting only of small, fine, high velocity droplets, was found on the sheet walls of the test chamber after the first cutting. In the second experiment, using a freshly killed pig, the pattern of spatter was similar; however, an increased volume of bloodier spatter was seen on the lateral walls. Both the victim and the two experimental carcasses showed characteristic striations across bony surfaces consistent with those seen on hard objects cut with a chainsaw. In all instances of dismemberment, some of the skin surfaces adjacent to the dismemberment sites were relatively smooth while other areas showed somewhat regular skin tags. The plywood sheeting that supported the pig in the first dismemberment showed superficial divots similar to those seen at the scene.

Detailed results will be presented of the experimental reenactments of the postmortem dismemberment. The characteristic patterns associated with blood and tissue spatter, as well as, tool mark impressions on bones and floor will provide insight into potential cases in which a successful dismemberment has occurred.

**Chainsaw Dismemberment, Tissue Spatter, Tool Marks**

## A12 A Preliminary Study: Evaluating the Error Rate Associated With Bloodstain Pattern Analysis

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After attending this presentation, attendees will be able to evaluate the error rate associated with basic bloodstain pattern recognition.

This presentation will impact the forensic community by illustrating the first systematic attempt at determining an error rate for bloodstain pattern analysis. This information can be critical in evaluating if bloodstain pattern analysis can be used in court.

Over the past 25 years, the individualization of biological evidence has improved dramatically. Forensic DNA testing has literally transformed forensic science. Since the early days of Gross and Locard, forensic science was an active part of the investigation. Over time the laboratory seemed to drift more into a reactive role primarily being used at trials. The power of DNA databases changed that. Now once again, forensic science could be used to further the investigation and even develop suspects. As useful as forensic DNA testing has become, it is not a panacea. A blood sample collected from a pool under the copiously bleeding victim will most likely be from that victim.

Since all of the bloodstains on a case are not usually tested, understanding the basic mechanisms of bloodstain pattern formation is necessary to adequate sample evidence for DNA testing. Also there can be times when understanding bloodstain patterns can provide more information than the subsequent DNA analysis. When a suspect claims that they received the victim's blood on their clothing after they attempted to help them, this issue cannot be resolved with DNA. The suspect already admitted that it was the victim's blood. In this case the pattern produced by the blood can be more useful than the knowledge of whose blood it is.

The information provided from bloodstain patterns have been used in criminal investigations and court rooms since the late 1800s. As scientific evidence, it has been subject to the different admissibility standards that have evolved in the US criminal justice system. The *Frye* standard (Frye v. United States, 54 App. D.C. 46, 293 F. 1013, 1014 (1923)) essentially evaluated if the expert was qualified, if their testimony will assist the jury, and if the science used was generally accepted by the scientific community. For the most part Bloodstain pattern testimony has not undergone a significant challenge through the Frye standard.

In the early 1990's a civil case reevaluated scientific expert testimony. Daubert v. Merrel Dow Pharmaceuticals, Inc., (509 U.S. 579 (1993) changed the admissibility of scientific evidence to include a more detailed evaluation of the methodology used in the analysis. *Daubert* asserted that the procedure used for the analysis had been tested, subject to peer review, have a defined error rate, and/or was generally accepted.

Not every state uses the *Daubert* standard. Some use Fry or even a combination of Frye and Daubert. For the states that are affected by *Daubert*, techniques such as bloodstain patterns must demonstrate an error rate which can be challenging to quantify. This preliminary study evaluated the error rate associate with the first step of bloodstain pattern analysis, basic pattern recognition.

To accomplish this, known bloodstain patterns were created in a controlled environment using defibrinogenated sheep's blood. The patterns were then photographed with a scale in place using a digital camera. The resultant images were then incorporated into a web-based survey tool. To eliminate issues of stain terminology, participants were asked to describe in a text box how the patterns were produced.

Once all of the patterns were produced, an alpha test was performed with the known patterns and a number of different qualified bloodstain pattern analysts in the same web-based format. These analysts ranged from crime scene personnel to scientists, both with a history of significant publication in the area of bloodstain pattern analysis. In order for a question to remain on the survey, it had to be answered correctly by 100% of the individuals taking the alpha test.

The final version of the test was given to participants that were directly solicited based upon directories to professional organizations and other means. While the survey was performed anonymously, a generic password was used to gain access. Once on the site, some basic information was collected about the participants: education, training, professional affiliations, certifications, and experience. A text box was also provided for participants to add any additional information that they thought was pertinent. Before beginning the study, participants watched a very brief video that described the significance of the study, how the study was created and how the patterns should be evaluated. After the pattern recognition portion of the survey was complete, a brief questionnaire followed about the survey.

**Daubert**, **Bloodstain Patterns, Error Rate**

## A13 Human DNA Extraction and Identification From Feces

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After attending this presentation, attendees will understand the criteria for selection of a DNA extraction protocol for human fecal evidence samples that affords the best chance of obtaining a complete genotype profile with the least amount of allelic dropout or degradation.

This presentation will impact the forensic community by comparing the advantages and pitfalls associated with using either the QIAGEN QIAamp® DNA Stool Kit or the BioRobot EZ1 Workstation® for processing human fecal evidence for forensic STR genotype analysis.

Careful consideration and selection of the extraction methods will determine the ability to generate a useful genotype profile, and the choice will depend on awareness of the technical issues and nuances of extracting DNA from fecal samples associated with silica-based manual extraction or automated protocols using magnetic bead technology.

The purpose of this study is to compare the QIAGEN QIAamp® Stool Mini Kit with the BioRobot EZ1 Workstation® for DNA extraction-STR genotyping of human feces. Direct sampling (excision) or swabbing of fecal samples was also compared to determine the most efficient method to generate an optimal amount of DNA. Earlier research by others suggested that epithelial cells, which are able to be isolated for DNA extraction, remain on the outer surfaces of a fecal sample and although DNA can be successfully extracted it was often inhibited during

the amplification process due to bacterial DNA, DNAses, bile salts and/or polysaccharides. Due to this known inhibition the QIAamp® kit utilizes a proprietary InhibitEX® tablet and buffers which allows for the removal of degradative enzymes and inhibitory substances. Because of this dilemma, a modified protocol has been developed for use with the QIAamp® kit to help overcome this PCR inhibition.

It is thought that extraction using the modified protocol will yield more quantifiable and uninhibited, human DNA than the original protocol (QIAGEN QIAamp® Stool Mini Kit) or the use of the BioRobot EZ1 Workstation®. Fecal samples for DNA extraction were obtained using either an excision of ~200mg or a complete swabbing of the outer, fecal "cellular" layer. Samples were then quantified by Applied Biosystems Quantifiler™ method using real time PCR. The original protocol using the QIAamp® kit resulted in the overall highest quantities of amplifiable DNA. Selected samples were concentrated and recovered using a Microcon® 100, then STR-typed by Applied Biosystems Identifiler™ utilizing an Applied Biosystems Prism 3130 Genetic Analyzer. It was learned that those extracted with the stool kit gave the most complete profiles with the least amount of allelic dropout or degradation. Samples which showed inhibition during quantification were successfully amplified after addition of bovine serum albumin.

In conclusion the best possible way to extract quantifiable DNA from feces without concern for downstream PCR inhibition is to use the Qiagen QIAamp Stool Mini Kit with the original protocol. Certainly, it should be realized that each person varies in the amount of exfoliated epithelial cells, the choice of DNA extraction methods may not be that crucial. Crime laboratories also need to consider time and cost factors, so that it my end up being more efficient to use the system already in place, such as the BioRobot Workstation. Ultimately in the forensic community, the efficient extraction of DNA from forensic samples will lead to successful identification which is the primary goal.

**Real Time PCR, Inhibition, Feces**

## A14 Population Studies and Proposed Nomenclature for 17 Equine STR Loci for Forensic Purposes

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The goal of this presentation is to describe how repeat-based nomenclature is highly relevant to the field of animal forensics.

In recent years the horse industry has become a fast growing business, forensically relevant cases involving horses, such as identification of samples involved in doping control, fraud or horse theft, are relatively common. Fourty-three breeds consisting of a total of 42000 horses were genotyped using 17 microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX3, and VHL20). To assess the power in kinship analysis and identity testing, the Power of Exclusion was calculated for 1 and 2 parents (PE (1) and PE (2)), the Expected Heterozygosity (HE), the Observed Heterozygosity (HO), probability of identity (HW P(ID) and sib P(ID)) and null allele frequencies for the 17 markers. When using microsatellite markers for equine kinship analysis, major differences exist in the reliability of the test between different horse breeds. To assess the variation between breeds, the genetic distances were calculated using Reynolds' distance Fst and Nei's standard Gst. In general, the genetic distances of the current study were similar compared to those estimated in earlier horse diversity studies. The power of individual assignment tests was assessed using the seventeen markers. Finally, based on sequencing of the most frequent alleles in the population, we propose a repeat number -based nomenclature for the 17 STR loci.

**Equine Short Tandem Repeats, Repeat-Based Nomenclature, STR Population Studies**

## A15 Evaluation of a New DNA Extraction Kit for Degraded Skeletal Remains

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After attending this presentation, attendees will understand information and test results from a new DNA extraction kit on skeletal remains.

This presentation will impact the forensic community by providing a simplified procedure for bones and teeth that doesn't require powdering, reduces time, and potential contamination and yields full STR profiles from highly degraded samples.

A new DNA extraction kit for degraded skeletal remains was designed and tested. Based on a method originally developed at Shinshu University in Japan (Fukushima et al. 2006), the new extraction kit has been utilized to extract DNA that yields complete STR profiles from degraded skeletal remains. The method significantly simplifies most skeletal extraction procedures, as it does not require powdering. In addition to saving time, this new method reduces the possibility of contamination. Samples, such as teeth, remain physically intact after the extraction and therefore retain their morphological evidentiary value.

Both STR and mtDNA have been analyzed from DNA extracted from skeletal remains using the new extraction method. Furthermore, the new kits require no additional specialized equipment or instruments.

**Reference:**

Fukushima, H. et al 2006. High-yield method of DNA extraction from old and degraded samples of human skeletal remains. 17th International Symposium on Human Identification. Poster abstract 74 http://www.promega.com/geneticidproc/ussymp17proc/abstracts/Abstract74Fukushima.pdf

**DNA Extraction Kit, Bone, Degraded**

## A16 Development of a One-Step Cell Elution and Preferential Lysis Method for Analysis of Sexual Assault Samples

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After attending this presentation, attendees will gain understanding of the improvements that have been made to the elution of cellular material from cotton swab samples collected from sexual assault victims and preferential lysis of epithelial cells recovered from the swab.

This presentation will impact the forensic community by describing an alternative, faster method, for increased cell recovery and comprehensive epithelial cell lysis from a vaginal cotton swab, than conventional differential extraction, providing the potential to shorten analysis times for sexual assault samples.

Differential extraction is a widely accepted method for the separation of male and female cellular material recovered from sexual assault swabs in order to obtain profiles of both the victim and the perpetrator. Differential extraction is laborious and time consuming, sometimes requiring an overnight incubation of the swab. This method utilizes proteinase K and a detergent to elute the majority of cellular material and selectively lyse epithelial cells. While this method has been shown to comprehensively lyse epithelial cells, some sperm cells are lost due to proteolytic digestion, thus decreasing the probability of obtaining a profile of the perpetrator in subsequent STR analysis.[1]

The use of microdevices for forensic analysis provides rapid, cost-effective alternatives to traditional DNA analysis methods. In addition, DNA analysis steps can be integrated onto one microfluidic device which will reduce analysis time and, as a result, the current backlog of casework samples. The separation of sperm cells and female epithelial cell lysate using a microfluidic device has been recently demonstrated[2]; however, this method is dependent on the recovery from the cotton swab of sperm cells as well as comprehensive lysis of epithelial cells from the vaginal lining.

Previous studies have shown that anionic detergents considerably increase the percent recovery of sperm cells over a conventional DE buffer.[1] A two step method for cell elution and preferential lysis of epithelial cells has recently been presented, which first elutes the cellular material then preferentially lyses the female epithelial cells while still obtaining high recovery of sperm cells.[3] This method uses a thirty minute incubation for cell elution and a second thirty minute incubation for preferential lysis of the epithelial cells. Using the results obtained from these studies, a one-step cell elution and preferential lysis method has been developed, which uses an anionic detergent, proteinase K, and an Orchid Cellmark reagent in the elution buffer. This improved method leads to cleaner slides for sperm counting and aids in the production of cleaner male profiles following DNA extraction.

Current work focuses on optimizing this one-step cell elution and preferential lysis method for sexual assault swabs. Several elution and lysis buffer components were evaluated along with different incubation conditions to determine the optimal recovery conditions. The sperm and epithelial cells eluted from mock sexual assault evidence swabs were counted using a hemacytometer. The results indicate that this one step method has comparable sperm cell recovery to the previous two step method and utilizes a much shorter incubation period, as little as thirty minutes. This method could be used in conjunction with conventional DNA analysis steps or eventually integrated in a lab-on-a-chip device for total DNA analysis.

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3 Norris, JV, Cunniffe, H, Manning, K, Linke, SJ, Ferrance, JP, Landers, JP. Development of an Improved Cell Elution and Preferential Lysis Method for Sexual Assault Cotton Swab Evidence Analysis; Washington, DC. American Academy of Forensic Sciences, 2008 Feb 18-23.

**Cell Elution, Preferential Lysis, Differential Extraction**

## A17 Using SNPs to Predict Hair Pigmentation in Individuals of European Ancestry

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After attending this presentation, attendees will become more familiar with genetic methods for predicting hair pigmentation. The genetics of human hair pigmentation will be briefly reviewed, as well as its forensic utility. In addition, the effectiveness of SNP analysis in predicting hair pigmentation in individuals of European ancestry will be discussed.

This presentation will impact the forensic science community by discussing an assay for inferring the lightness or darkness of an individual's hair color. Data from such an assay could be useful in legal investigations.

An assay that could help provide a physical description of a person from a DNA sample would be helpful when eyewitnesses are either not available or have conflicting reports. In addition, such an assay could help identify missing persons who cannot be identified through traditional means. On average, medical examiner and coroners' offices handle roughly 4,400 unidentified missing persons each year, with approximately 1,000 remaining unidentified after a year.[1] In these instances, a lack of reference DNA can hamper a successful identification. To address this, researchers have investigated genetic loci that produce phenotypic differences among individuals. This could aid in identifications by producing a physical profile of an individual. Thus far, assays have been developed to predict an individual's eye color,[2] ancestry,[3] and red hair color[4] using single nucleotide polymorphisms (SNPs) in conjunction with population allele frequencies. Since the existing hair pigmentation assay can only differentiate red and non-red, it would be useful to have an assay to predict other hair colors.

Hair pigmentation, along with skin and eye pigmentation, is determined by melanin. There are two types of melanin; eumelanin is the black/brown pigment, while pheomelanin is the yellow/red pigment. Melanin is synthesized in melanocytes, and the number, size, and distribution of melanocytes contribute to the shade differences observed

in hair, skin, and eyes.[5] Strong correlations exist between hair and/or eye color and the SNPs examined. Therefore, the genotypes of each SNP are expected to correlate with hair pigmentation.

Ten SNPs located in nine pigmentation genes were chosen for analysis, including *SLC24A4*, *KITLG*, *OCA2*, *TYR*, *IRF4*, *MATP* (*SLC45A2*), *HERC2*, *TYRP1*, and *SLC24A5*. DNA samples were collected, along with data on background characteristics (including hair pigmentation) and ancestry informative markers. Two primer extension multiplexes were developed and optimized for genotyping the samples. The genotypes were tested for linkage to hair pigmentation by admixture mapping, and a model for predicting hair pigmentation was designed.

The proposed model was then tested by analyzing an additional set of samples blind. The results were compared to the individuals' original reported hair color and pigmentation measurements, and the accuracy of the chosen SNPs in predicting hair pigmentation was determined. The effectiveness of the ten SNPs individually, along with interactions between pigmentation and ancestry, were determined

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**Hair Pigmentation, DNA, SNP**

## A18 Analysis of Oxidative Damage DNA in Degraded Tissue of Forensic Samples

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The goal of this presentation is to develop a method for evaluate the concentration of 8OHdG in DNA from both degraded and undegraded tissue samples, using HPLC with UV - EC detection.

This presentation will impact the forensic community by comparing the levels of 8OHdG in various tissue samples in order to develop a biomarker useful in assessing sample quality for subsequent PCR amplification.

In postmortem decay, the single most important factor in the degradation of DNA is the rate of which cellular nucleases degrade the endogenous DNA. However, the DNA recovered from tissue in such samples may also be heavily degraded through hydrolytic cleavage and oxidative base damage, limiting its successful retrieval and amplification. The major site of oxidative attack on the DNA bases are the C=C double bonds of pyrimidines, and purines, leading to ring fragmentation and base modifications. A majority of these oxidative base products are replication blocks and this process will affect amplification with standard Taq-DNA polymerases used in PCR.

Modified purine and pyrimidine bases constitute one of the major classes of hydroxyl-radical-mediated DNA damage. Guanine nucleobases are frequently targeted by oxidants due to their lowest oxidation potential among the DNA bases. Among guanine oxidation products, 8-oxo-7,8-dihydro-2´-deoxyguanosine (8-OH-dG) is used widely as a biomarker for guanine oxidation because of its *in vivo* incidence and its facile measurement by HPLC with electrochemical detection. Specifically, 8-OH-dG is know to cause GC →TA transversions and its presence in DNA causes mutations resulting in mispairing and multiple amino acid substitutions.

Levels of 8OHdG in cells, tissue, and whole animal have been reported as an important biomarker for oxidative stress when evaluating disease pathologies. Thus it is likely that this same compound may provide information on the relative amount of oxidative damage to target tissues used in forensic STR and mitochondrial analysis. Such an assay would be useful in situations where it is not clear if the lack of amplification success is the result of PCR inhibition, oxidative damage or fragmentation. The aim of this study will be to develop a method for evaluate the concentration of 8OHdG in DNA from both degraded and undegraded tissue samples, using HPLC with UV-EC detection.

This work will describe the application of enzymatic hydrolysis of DNA and HPLC-UV-EC detection methodology for the determination of 8OHdG in forensic samples. The goal of the study is also to compare the levels of this compounds in various tissue samples in order to develop a biomarker useful in assessing sample quality for subsequent PCR amplification.

**Oxidative DNA Damage, HPLC-UV-EC, 8OHdG**

## A19 DNA Degradation in Simulated Arson Cases Using Various Accelerants

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After attending this presentation, attendees will learn the results of systematic experiments using three accelerants, including methyl ethyl ketone, gasoline, and a mixture of lighter fluid, gasoline, and diesel fuel, on the quality and quantity of DNA recovered as assayed using UV-Vis spectroscopy and agarose gel electrophoresis from controlled burns using pig muscle to simulate a potential cases resulting from an accidental fire, arson fire or a mass disaster event. In particular, each one of these three accelerants exhibit different burn rates and temperatures and affect the quality and quantity of DNA recovered after various time intervals.

This presentation will impact the forensic community by providing systematic data that can be used in evaluating cases of accidental fire, arson, and mass disaster. Both agarose gel electrophoresis and UV-Vis spectroscopy methods allow an investigator to determine the presence and quality of DNA samples recovered from the crime scene using rapid and non-destructive techniques. The determination of which samples provide quality DNA in comparison to those that yield no detectable DNA may help the investigator to decide which samples to collect and package for further DNA processing and which are less likely to produce results.

The use of DNA to identify human remains after an accidental fire, arson fire or even a mass disaster has become a cornerstone in the forensic community. This presentation involves the use of three prevalent

accelerants that cause a fire to proceed at a much faster rate and at a higher temperature and how the use of each one can have a unique effect on tissue and bone. When dealing with arson victims and the need to identify burned remains, it has not been confirmed when autosomal and mitochondrial DNA extraction should be used. There is a definitive window of time, dependent upon the accelerant used and length of time of burn, when autosomal STR analysis of DNA can be used to identify a burn victim. This research sought to answer how much time it takes to burn a piece of flesh to the point that DNA cannot be extracted used for identification purposes based on quality and quantity, if the accelerant itself causes degradation to the DNA when it is applied to the flesh prior to the burn, and if any initial degradation causes the appearance of heightened burn degradation in order to determine the window of time available and to suggest a protocol to analyze the DNA available.

The research described in this presentation includes both the detailed systematic methods constructed in this study and answers to the questions posed by concluding the results of each controlled burn including both agarose gel (0.8%) electrophoresis and UV-Vis spectroscopy (260/280 nm ratio) results. Standard DNA extraction were used techniques (phenol/chloroform) using pig muscle as purchased from the supermarket. In order to determine the effect of accelerants on DNA, one large rack of pork ribs was divided evenly and each piece was analyzed individually using an equal proportion of accelerant by mass. The accelerants used were methyl ethyl ketone, gasoline, and a mixture of lighter fluid, gasoline, and diesel fuel. Control samples of unburned and unexposed pig were also assayed. A known amount of accelerant was applied to each piece being analyzed and a second sample was taken to determine whether degradation had already started to take place. A controlled burn was done in the laboratory and three pieces of pig were burned for various times with each accelerant. Additional samples of DNA were taken from different areas of each piece and analyzed to confirm the initial results.

**Arson, DNA, Accelerants**

## A20    DNA Extraction and STR Typing of Compact Bones from Decomposed Human Skeletal Remains by Using Decalcification Treatment

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The goals of this presentation are to investigate the effects of decalcification treatment using 0.5 M EDTA - 3K on DNA typing of compact bones from decomposed human skeletal remains. After attending this presentation, attendees will understand that decalcification treatment with 0.5 M EDTA - 3K improve the success of genomic DNA typing in identification of decomposed human skeletal remains.

This presentation will impact the forensic comminuty by demonstrating the decalcification treatment with 0.5 M EDTA -3K improved genomic DNA recovery and results in higher detatability than undecalcification process during DNA

Multiplex PCR-based STR analyses are suitable in human identification and forensic casework dealing with different tissues, even when the sample is heavily decomposed. The extraction of DNA from forensic skeletal remains can provide quite powerful data for analysis, but is plagued by a unique set of methodological problems. Bone is the most resistant tissue in deceased bodies to time depending degradation and putrefaction, but it is often hard to extract DNA from it due to its highly mineralized structure, which makes DNA extraction and/or purification hard to carry out. DNA extraction was performed and STR typing of decomposed human skeletal remains by using both undecalcified and decalcified methods. The postmortem periods of the studied remains ranged from two weeks to eighteen years. In some cases, they were buried with exhumation.

Decalcification using 0.5 M EDTA-3K at 56 overnight and repurification were used prior to the digestion and extraction to overcome inhibition of amplification process. DNA was isolated using standard phenol/chloroform/isoamyl alcohol extraction. This study detected human DNA in 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) from skeletal remains using the Applied Biosystems' kit. Standard phenol/chloroform/isoamyl alcohol extraction followed by decalcification method has been proved as the most successful method. Decalcification with 0.5 M EDTA-3K was shown to improve the success of DNA typing in this study. A duo case, the Combined Paternity Index (CPI) value of the 15 STR loci from decalcified bone sample was higher than undecalcified bone sample in the paternity testing (99.677 % / 99.997 %). This study demonstrated that DNA extracted from highly decomposed bony tissues of human remains up to eighteen years old by using decalcification treatment was successfully amplified and greatly increased our ability to positively identify previously unknown skeletal remains by a comparative genetic analysis with presumative relatives.

**Decalcification, Human Skeletal Remains, Combined Paternity Index**

## A21    Sequencing of Select Novel X Chromosomal Short Tandem Repeat Alleles

*Toni Marie Diegoli, MFS*, and Mike Coble, PhD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Rockville, MD 20850*

The goal of this presentation is to describe allele sequencing results from a select set of short tandem repeat (STR) markers located on the X chromosome. The presence of new alleles and microvariants previously not observed in published population data will be presented. The attendee will also learn about the process of allele sequencing, from sequencing primer design to sample selection, and data analysis.

This presentation with impact the forensic community by presenting data illustrating both the sequences of the new alleles in comparison to published allele sequencing data as well as information on the prevalence of these novel alleles in the U.S. populations examined.

The multiplex detection and analysis of STR markers is a common tool used for genetic identity testing in the forensic setting. Numerous publications have characterized genetic markers located throughout the autosomes and male-specific Y chromosome. More recently, markers located on the X chromosome have emerged as additional tools in this forensic arsenal. X chromosomal STRs can be used to supplement traditional kinship testing due to their unique inheritance pattern and, correspondingly, the breadth of published literature on the subject has expanded greatly in recent years due to this increasing interest in their utility. Numerous X STR markers have been characterized and a variety of multiplexes proposed. Currently only one commercial kit for X chromosomal STRs is available, and it is in use predominately throughout Europe. The process of allele sequencing is a necessary part of this

growth in available information because it is able to reveal the molecular basis for the variation seen in these markers and aid in understanding the observed STR results.

Because DNA templates encountered in the forensic setting, and at the Armed Forces DNA Identification Laboratory (AFDIL) specifically, are often degraded, amplicon size should be considered in selecting potential markers for use in the forensic laboratory. In such cases, shorter amplicon sizes are favored with the goal of recovering the maximum number of alleles. Here, two reduced amplicon size multiplex STR (or mini-STR) assays were developed to type a total of 14 markers for 800 U.S. population samples from the five major subgroups: African American, Asian, Western European Caucasian, Hispanic, and Native American. Through this databasing process, the authors noted several novel variants that were present in these population samples and not previously reported in the literature. In most cases, published primer sequences were used in these two multiplexes, but in two instances, alternative amplification primers were designed to achieve the smaller amplicon size desired. Sequencing of these new alleles was performed to verify concordance of the repeat structure with that of the published data obtained using larger amplicons. Additionally, the sequencing process was used to investigate the presence of potential microvariants observed in the data. For seven markers in particular – DXS9902, DXS7423, DXS7424, DXS7130, DXS7132, DXS7133, GATA31E08 – new alleles and/or microvariants were confirmed.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

**Mini-STRs, X Chromosome, Allele Sequencing**


## A22     The Evaluation of Eight Commercially Available STR Kits

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After attending this presentation, attendees will have a better understanding of the performance of eight commonly used STR amplification kits.

This presentation will impact the forensic community by providing information on the key performance measures of each amplification kit enabling the laboratory to make more informed decisions when choosing the kit(s) that best suit their needs.

A number of commercially available short tandem repeat (STR) amplification kits are available for use in forensic DNA laboratories. While it is the responsibility of each laboratory system to evaluate and choose the analytical methods that best suit its needs, it is important that forensic DNA analysts have a general understanding of the performance of commonly used STR amplification kits. In an effort to assist in this process, the National Forensic Science Technology Center (NFSTC) conducted a study to evaluate the performance of eight STR amplification kits: Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit, Yfiler® kit, Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system.

The performance of each STR amplification kit was assessed based on a defined set of criteria: sensitivity, peak ratios at heterozygous loci, baseline noise, stutter ratio, and amplification artifacts. These criteria were determined through the analysis of single source human DNA samples. A mixture series was prepared and analyzed to assess the percent contribution necessary to detect a minor contributor in a two donor mixture for each STR amplification kit.

Two separate known human DNA standards were prepared utilizing a standard organic extraction method in conjunction with the Millipore Microcon® 100 centrifugal filter device. The samples were quantitated using the Applied Biosystems Quantifiler® Human and Y Human Male DNA Quantification Kits on an Applied Biosystems 7500 Real-Time PCR System. To minimize variation, a large volume (1000μl) of each sample was prepared and used for the dilution and two person mixture series. Samples were amplified on an Applied Biosystems GeneAmp® PCR 9700 thermal cycler following manufacturer's specifications. The samples were then separated and detected using an Applied Biosystems 3130*xl* Genetic Analyzer and the data was analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 rfu.

A serial dilution was performed on a known human DNA sample to yield target concentrations of 1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078 ng. The data obtained from each of these samples was used to assess the sensitivity and peak ratios at heterozygous loci for each of the eight STR amplification kits. In addition, the observation of any reproducible amplification artifact(s) in the data was noted. The baseline noise was assessed by evaluating the data from ten injections of amplification negative controls for each of the eight STR amplification kits.

A two donor mixture experiment was performed to evaluate the percent contribution necessary to detect a minor contributor for all eight STR multiplexes. Two separate known human DNA standards were systematically combined to create the following mixture ratios: 1:20, 1:15, 1:12, 1:10, 1:8, and 1:5. The DNA profile from the minor contributor was evaluated at a 75 rfu threshold and the peak ratios at heterozygous loci was calculated and noted at all loci where the alleles were 75 rfu or higher.

There are various commercially available STR multiplex kits available to the forensic science DNA community that are designed to address the ever changing needs of crime laboratories. A primary goal of this study is to provide an overview of key performance measures of these eight commercial available STR kits. The demand on forensic DNA laboratories to employ methods that meet their system's needs is a continual challenge and is compelling DNA technical leaders and laboratory management to acquire relevant information that will aid in making these crucial decisions.

**Kit Comparison, Amplification Kits, STR Kits**


## A23     Using DNA Analysis to Assist in the Investigation of Stolen Vehicles

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After attending this presentation, attendees will understand the investigative value of DNA evidence in recovered stolen vehicles and which types of evidence generate the best results for CODIS entry.

This presentation will impact the forensic community by showing the investigative value of DNA evidence in recovered stolen vehicles.

In 2007 Las Vegas was ranked the number one city in the country in auto thefts per capita by the National Insurance Crime Bureau. At this

time there was a particularly high number of Honda vehicles being stolen. In an attempt to combat this growing problem, members of the Las Vegas Metropolitan Police Department Forensic Laboratory's Biology/DNA Detail collaborated with the department's Auto Theft and Crime Scene investigators to target the collection of DNA swabs from areas of the vehicles where thieves would most likely touch: steering wheels, gear shifts and rear view mirrors. In addition, if there were any personal items left in the vehicles that did not originate from the owners, those items were also submitted for DNA analysis. Because detectives often have no tangible leads to begin investigating suspects involved in an auto theft, a DNA profile eligible to be entered into CODIS could help solve these cases.

Over 200 evidence samples were collected from 87 recovered stolen vehicles. Approximately one-third of these samples were swabs collected from steering wheels, one-third were swabs collected from gear shifts and rear view mirrors and the remaining one-third were personal items left in the vehicles and other areas with obvious biological evidence such as blood. Reference samples were only available from sixteen of the vehicle drivers and three possible suspects. All evidence samples were extracted using organic methods, quantitated using real-time PCR, amplified with a fifteen locus STR amplification kit and analyzed using capillary electrophoresis. Reference samples were processed similarly with the exception of being extracted using non-organic extraction methods.

The majority of the DNA profiles obtained from the swabs collected from the steering wheels, gear shifts and rear view mirrors were consistent with mixtures of at least three individuals. With very few reference standards available from vehicle drivers a putative perpetrator profile was not able to be deduced for searching in CODIS. Approximately one-fourth of samples produced inconclusive or no DNA profile results. However, the majority of personal items left in the vehicle yielded single source DNA profiles eligible for upload to CODIS.

Based on the results of the study, it was concluded that personal items and obvious biological evidence yield the highest success rate in generating CODIS eligible profiles. Without the submission of vehicle owner standards, DNA mixture profiles obtained from steering wheels and other car parts are of limited investigative help.

**DNA, Auto Theft, CODIS**


## A24 Improving Traditional Multiplex STR Amplification of Low Template DNA Samples With the Addition of Proofreading Enzymes

*Carey P. Davis, BS\*, Lynzee A. Chelland, María José Illescas, BS, and Tracey Dawson Cruz, PhD, 1000 West Cary Street, PO Box 842012, Virginia Commonwealth University, Richmond, VA 23284*

After attending this presentation, attendees will become aware of alternate less expensive methods for improving STR profiles from low template DNA samples using proofreading enzymes in combination with Taq polymerase for multiplex STR amplification.

This presentation will impact the forensic community by potentially providing a faster, cheaper alternative method for generating STR profiles from low template and/or challenged biological evidence samples. This approach includes the addition of no new analytical steps and could be accomplished with very little additional cost to the laboratory.

Touch or trace DNA evidence, including fingerprints, saliva, hairs, and miniscule drops of blood and other bodily fluids are sometimes the only evidence found on a crime scene. This type of evidence can often contain less than 100 pg of DNA (~15 diploid cells or less) and is referred to as low template DNA evidence. Because of the limited quantity of DNA available, these types of samples can become difficult to analyze and interpret with traditional STR analysis, preventing the acquisition of a full or even strong partial profile. With <100pg of template DNA, stochastic effects often prevail, including allele dropout, inter- and intra-locus peak imbalance, and high stutter. To overcome these limitations, some researchers have investigated preamplification methods that include the addition of proofreading enzymes to the PCR cocktail. Proofreading enzymes have 3'-5' exonuclease activity, allowing them to correct bases that were misincorporated by the traditionally used *Taq* polymerase. Typically, the addition of an enzyme that has proofreading capability results in longer fragments, although the exonuclease activity reduces the overall processivity of the reaction. Previous studies have shown that combining these proofreading enzymes with *Taq* polymerase for preamplification is the best approach for increasing fragment length *and* STR genome coverage, without compromising the speed of the reaction. However, preamplification techniques, such as whole genome amplification (WGA), are often labor intensive and more costly than traditional STR analysis. Thus, this project will seek to determine if combining proofreading enzymes with *Taq* directly into the standard STR amplification reaction mixture will improve the fidelity of the reaction when little template DNA is available. This is vital for STR multiplex reactions because if longer products can be obtained, then the number of STR copies generated would increase, decreasing allele drop out and increasing the probability of obtaining a complete STR profile.

For this project, a series of STR amplifications will be conducted using input DNA quantities from 7.5pg – 100pg and various ratios of *Taq*: proofreading enzymes. Two enzyme combinations were tested including a *Taq*:*Deep Vent* combination and a mixture of Taq Gold and an unknown proprietary enzyme(s). These enzyme mixtures were used in place of Taq Gold for multiplex STR amplification. Resulting STR products were separated and analyzed via capillary electrophoresis. STR success was measured by percentage of alleles present, intra-locus heterozygous peak balance, and the occurrence of other stochastic effects. STR data obtained using proofreading polymerase combinations will be compared to data obtained using traditional STR amplification (with Taq Gold alone) as well as to STR data obtained using other methods designed for low template DNA analysis. STR data is being accumulated for analysis and all results will be presented and discussed.

**Low Copy Number DNA, Deep Vent, Proofreading Enzymes**


## A25 Forensic Utilization of Familial Searches in DNA Databases

*Linda C. Rourke, MS, MPhil, PO Box 610104, Bayside, NY 11361; and Cassandra Gershaw, BS\*, 25-37 72nd Street, 2nd Floor, East Elmhurst, New York 11370*

After attending this presentation, attendees will be familiar with: (1) the importance of DNA evidence with regard to the forensic utilization of DNA database searches, (2) the process of forensic familial searches in DNA databases, (3) the state-by-state code variations pertaining to

familial searches in DNA databases, (4) the potential positive and negative consequences of utilizing familial searches, and (5) case examples of successfully utilized familial DNA searches.

This presentation will impact the forensic community by increasing awareness of recent changes within the law regarding the utilization of familial DNA searches, as well as the potential for a higher number of closed cases if familial DNA searches are employed.

The technology of DNA evidence has become an invaluable tool in the process of identification and investigation, as well as the 'gold standard' on which juries rely during their deliberations of whether or not to convict. The development of state and federal DNA databases has greatly impacted the forensic community by creating an efficient, searchable system that can be used to eliminate or include suspects in an investigation based on matching DNA profiles – the profile already in the database to the profile of the unknown sample in evidence.

The importance of DNA evidence is widely recognized and largely irrefutable. Recent changes in legislation have begun to allow for the possibility to expand the parameters of DNA database searches, taking into account the possibility of low-stringency or familial searches. Throughout this presentation, the terms "familial" and "low-stringency" will be used interchangeably to discuss the forensic process of searching a DNA database based on matching only a limited subset of the available typed loci. Such searches would yield a larger number of possible suspects by incorporating near-hits – DNA samples that match the unknown sample on a fewer number of loci. These near-hits may indicate a close relative to the source of the unknown forensic sample, thereby broadening the inclusion criteria of the searched DNA database to include not only offenders, but also these offenders' relatives.

Acceptable uses for DNA database searches (as well as which DNA profiles are included in the database) are dictated by state codes which differ from state-to-state and are currently expanding. Recently, certain states (i.e., California) have begun to allow the process of low-stringency searches or have attempted "test runs" in an effort to identify the potential positive outcomes of allowing the utilization of familial searches (i.e., Colorado). In terms of the FBI-developed Combined DNA Index System (CODIS), familial searches are not in common practice but partial matches are occasionally acknowledged, and the decision to follow up on the possible involvement of a relative is left up to the state in question. The United Kingdom has been utilizing familial searching for the past few years and has already found success. Even in the United States there are examples of cases closed due to the use of low-stringency searches. For instance, the BTK killer, Dennis Rader, was identified through comparing forensic DNA evidence from the BTK case with a DNA sample obtained from a pap smear taken previously from Rader's daughter.

This presentation proposes to highlight the prospective importance of familial DNA searches by providing case examples in which familial searching was successful. This presentation will also acknowledge the possible negative outcomes of employing familial searches, thereby presenting both sides of this very complicated, rapidly evolving situation.

**DNA, CODIS, Familial Search**

## A26    Y - STR Haplotype Database Comparison for Colorado Residents

*Sylvia Thurmond, MFS\*, Bonnie Mountain, MA, and Susan Berdine, MS, Denver Police Department, FBIO/DNA Unit, 1331 Cherokee Street, Denver, CO 80204*

After attending this presentation, attendees will understand the importance of evaluating, supporting, and defending the selection of a publicly available Y-STR haplotype database for estimating haplotype frequencies.

This presentation will benefit the forensic community by providing an example of how to evaluate and determine the appropriate Y-STR haplotype database through the use of haplotype data collected from residents of a specific geographical region (in this case, Colorado).

The counting method is a simple and conservative method used to estimate the frequency of a Y-STR haplotype. This method relies on searching a specific Y-STR haplotype in a database of reference Y-STR haplotypes from unrelated, random individuals of self-described population groups (e.g., African American, Hispanic, and Caucasian) and utilizing the number of times that haplotype occurs in the database to estimate the upper bound frequency estimate using a 95% confidence interval. The width of the confidence interval is inversely related to the size of the database and may be used to estimate how often the specific haplotype would be expected to be observed in any given database (e.g., the entire US population). A number of web-accessible Y-STR haplotype databases are available to the forensic community (e.g., YHRD, Applied Biosystems, and Promega were publicly available at the time of this study). These databases vary in terms of size and searchable Y-STR loci. Due to the different database choices available to the forensic community, and to respond to challenges regarding the use of "pooled" versus "regional" databases, this study was designed to determine if the upper bound frequency estimate for Y-STR haplotypes generated from Colorado residents varied significantly when different haplotype databases were utilized.

At the Denver Police Department Crime Laboratory, Y-STR haplotypes were generated using the AmpfℓSTR® Yfiler® Y-STR PCR Amplification kit from 38 individuals who are employees of the Denver Police Department Crime Laboratory and are residents of Colorado. Complete 17-locus profiles were generated from all 38 samples, but for searching and comparison purposes, the 11-locus US Haplotype (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, and DYS439) was searched in the three available databases. The upper bound frequency estimate was determined for each searchable population group and the most conservative value (i.e., maximum upper bound frequency) was compared between the three databases for each haplotype. For 35 of the 38 Colorado samples, the Applied Biosystems database (N=3,561) provided the highest maximum upper bound frequency estimate and for 37 of the 38 Colorado samples, the YHRD database (N=22,999) provided the lowest maximum upper bound frequency estimate. These results are consistent with the view that the larger the database becomes, the more precise the frequency estimate is due to the width of the confidence interval decreasing. Therefore, due to the size of the database, the Applied Biosystems Yfiler® Haplotype database provided the most conservative upper bound frequency estimate for the majority of the Y-STR haplotypes searched. Also, it would be expected that the discriminatory power of the Applied Biosystems database is more powerful than this study reflects and a lower upper

bound frequency estimate would be obtained if all 17 Y-STR loci were searched in the database, rather than limiting the search to the 11-locus US Haplotype.

Since the completion of this study, the US Y-STR database was released and includes haplotype data from five different sources (i.e., National Center for Forensic Science, ReliaGene, Promega, Applied Biosystems and the University of Arizona) pooled into a single searchable database. Current work is underway to search this database with the same 38 Colorado 11-locus haplotypes to determine if this larger database is consistent with the previous results of this study. This study is expected to benefit the relevant forensic community by providing an example of how to evaluate and determine the appropriate Y-STR haplotype database through the use of haplotype data collected from residents of a specific geographical region (in this case, Colorado).

**Y-STR, Database, Comparison**

## A27 Micro-Manipulation and Isolation Techniques for the Collection of Spermatozoa From Smear Slides and Subsequent Analysis of DNA

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After attending this presentation, attendees will become aware of a more inexpensive, highly efficient, easy-to-learn workflow for processing rape victim evidence.

This presentation will impact the forensic science community by providing an alternative solution for achieving desirable PCR results when low numbers of spermatozoa are present.

The separation of spermatozoa from a mixture of assailant's spermatozoa and victim's epithelia should allow for a more certain identification of the assailant's DNA type. Although separation has been accomplished by a variety of other methods such as differential extraction[1] and by the use of microfabricated devices,[2] the direct isolation and pooling of individual spermatozoon has received little attention because of difficulty validating appropriate techniques and the cost of instrumentation such as that required for Laser Capture Micro-dissection. Furthermore, the other techniques that are used for separating spermatozoa and epithelial cells often reduce the number of spermatozoa recovered and thereby limit the amount of DNA available for typing.[3]

It has been estimated that 500pg (~ 150 spermatozoa) of DNA is required for reliable real-time PCR quantification.[4] On the other hand, fewer spermatozoa may be needed and more sensitive techniques devised if the spermatozoa can be isolated and cleaned of the contaminating epithelia. Mitochondrial or Pyrosequencing techniques were shown in this study to be more sensitive methods for characterizing and comparing the donor's DNA. Applying particle manipulating techniques used in McCrone Associates' laboratory for decades,[5] individual sperm were isolated from stained smears and transferred to sterile tubes for DNA analysis. Spermatozoa were isolated from both Kernechtrot-Picroindigocarmine (KPIC, Christmas Tree) stained smears and smears stained with Independent Forensics' Sperm Hy-Liter™ while employing fluorescence microscopy.

The smear is coated with a thin coat of water soluble adhesive (3M Water Soluble Tape) and each spermatozoon can be individually selected. While being observed with the microscope, the spermatozoon is picked from the slide surface with a finely pointed tungsten needle. The isolated spermatozoa are held intact by the adhesive and are transported to sterile tubes. Each pick requires approximately 10 seconds. The tubes are then processed for DNA. These isolation and manipulation techniques can easily be incorporated into an everyday screening process, and the amount of training that personnel would need in order to achieve desirable results is minimal.

Samples of spermatozoa were prepared for mitochondrial analysis as follows: 20 unstained, 20 KPIC stained and 20 Sperm Hy-Liter™ stained were transferred to 3 separate tubes; 40 unstained, 40 KPIC stained and 40 Sperm Hy-Liter™ stained were likewise transferred to 3 tubes. A negative control with the tube containing only soluble gum was processed with each batch along with six blank tubes, right out of the package. A saliva swab from the donor of the sperm was used as a reference sample.

DNA extraction using DTT, Proteinase K, PCIA, and PCR amplification were carried out to determine if mitochondrial DNA (mDNA) analysis could be performed on samples. The amplification target was a 281 base-pair fragment from the mDNA hypervariable region 1. Success of amplification was judged by a 1% agarose yield gel, and DNA sequencing was carried out to determine the mDNA profile of the sperm donor. A known buccal swab sample of the sperm donor was used to confirm that the correct profile was obtained. Negative extraction controls were amplified in parallel with the sperm to investigate whether the system for collecting sperm was free of contamination with exogenous DNA.

A similar set of samples were analyzed by Pyrosequencing (sequencing by synthesis) except that two additional duplicates were included. In other words, 3 tubes containing 20 picks from unstained, KPIC stained and Sperm Hy-Liter™ and 3 tubes with 40 picks from the same sources, making a total of 18 tubes plus controls, were prepared. Pyrosequencing data were obtained from as few as 20 spermatozoa. KPIC stained spermatozoa were more compatible with Pyrosequencing than Sperm Hy-Liter™ stained cells.

With hand micro-manipulation, spermatozoa can be isolated from the epithelial cells and collected from smear slides. Approximately 20 spermatozoa can be analyzed successfully for DNA. The need for expensive and time consuming digestion processes is eliminated, and one can achieve desirable DNA results with fewer spermatozoa on the smear.

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**DNA, Isolation, Sperm**

## A28 Obtaining STR Profiles From Low Copy Number Biological Materials Utilizing Laser Microdissection and Optimized Collection Procedures

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After attending this presentation, attendees will learn about the collection of low copy number samples using of laser microdissection for STR analysis.

This presentation will impact the forensic community by demonstrating that using laser microdissection with low volume amplifications, full STR profiles can be obtained in as little as five cells. Being able to precisely collect an exact number of cells or separate mixtures using laser microdissection and obtain full STR profiles may impact how many low copy number samples are processed.

Processing low copy number (LCN) samples for STR analysis is a challenging endeavor. In cases where low amounts of biological material are present and standard collection/elution methods are implemented, it is difficult to determine how many cells are recovered upon collection. In some cases, low levels of differing cellular types can be overlooked with standard screening methods resulting in STR mixtures. Commercially available quantification systems have difficulties with LCN samples due to low level sensitivity limitations which can lead to poor amplification results. With the use of laser microdissection (LM), we can visually confirm the presence/type of biological material, collect an exact amount of cells, and separate cells of differing morphologies to resolve mixtures.

Prior to utilizing LM, different techniques of cellular collection were investigated to determine the best collection method for the recovery of cells from paper, steel, and cotton substrates. Previous studies have shown that the enzymatic digestion of cotton swabs with cellulase improves elution of biological material. Based on these studies, we implemented and optimized the addition of *Aspergillus niger* cellulase and found improved elution of LCN biological material with no detrimental effects.

The collection of biological materials was performed on two separate laser microdissection systems. The first system utilizes laser energy and caps comprised of a thin thermoplastic film to remove tissues or individual cells. After the cell of interest is targeted with the laser, the laser is fired, melting the thermoplastic film on the base of the cap to the targeted material. The second system uses a high energy UV laser to transfer cells from glass slides into collection vessels via non-contact cellular catapulting. The laser utilized in this system first makes direct contact with target cells and then pressure catapults them into collection caps. Both of these systems allow analysts to collect an exact number of cells with extreme precision to carry through DNA extraction.

Several commercially available DNA extraction kits were evaluated for extraction efficiency from laser microdissected tissues. DNA extracts were concentrated to 3µl to allow for maximum template input for low volume amplifications. Amplifications were performed at 30-32 cycles using autosomal, mini-STR, and Y-STR multiplex amplification kits.

Using the optimized techniques listed above, we have achieved full STR profiles from 5 to 25 laser microdissected white blood cells, epithelial cells, and spermatozoa taken from paper, steel, and cotton surfaces. Single source profiles have also been obtained from two person mixtures from cells of different morphologies in as little as five cells. The use of LM has allowed us to determine the exact number of cells needed

obtain full STR profiles with various kits which eliminates setting up PCR reactions based on quantifications with LCN sensitivity limitations. The success of this study has shown that laser microdissection can be a powerful forensic tool for the precise collection and processing of low copy number biological evidence.

**Laser Microdissection, Low Copy Number DNA, Short Tandem Repeat DNA Typing**

## A29 Automated Searching of Ignitable Liquids Database by Summed Ion Spectra

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After attending this presentation, attendees will understand this method efficiently utilizes the information contained within a gas chromatographic-mass spectral data file. The gas chromatographic - mass spectral data files contain data used to create a summed ion spectrum which retains sufficiently high information content that it can be used for automated database searches of complex mixtures such as ignitable liquids.

This presentation will impact the forensic community by providing a complementary method to current fire debris analysis methods which follow ASTM E1618 standard method. The method of summing the intensity of each ion across the entire chromatographic range allows for rapid automated searching against a database of ignitable liquid spectra and a measurement of similarity between the ignitable liquid summed ion spectra. Furthermore, background "matrix" effects can sometimes be accounted for to aid in recognition and identification of ignitable liquid residues.

In fire debris analysis, the chromatographic patterns of total ion and extracted ion chromatograms are used to classify an ignitable liquid according to the ASTM E1618 standard method. A complementary method of summing the intensity of each ion across the entire chromatographic range allows for rapid automated searching against a database of ignitable liquid spectra and a measurement of similarity between the ignitable liquid summed ion spectra. Furthermore, background "matrix" effects can sometimes be accounted for to aid in recognition and identification of ignitable liquid residues.

Summed ion spectra were created from existing GC-MS data files obtained in the Ignitable Liquid Reference Collection (ILRC) database. Similarity comparisons between the normalized summed ion spectrum of an ignitable liquid sample and a database of ignitable liquid summed ion spectra were performed by custom software written in-house. The automated search produces a list of database entries and their similarity with the ignitable liquid sample in rank order from most similar to least similar. A similarity measurement between the summed ion spectra of 62 ignitable liquids was calculated. Cluster analysis based on the Euclidean distance between the similarity measurements was performed to determine if ignitable liquids within the same ASTM E1618 classification, as assigned by the Ignitable Liquids Reference Collection Committee, would cluster together. The ability of the database search method to correctly identify an ignitable liquid, its ASTM class and sub-class was evaluated by receiver operating characteristic (ROC) analysis. Since none of the time related data is retained within the summed ion

spectrum, it was proposed that summed ion spectra of the same ignitable liquid analyzed by different analytical methods and instruments could be identified, provided that both analytical techniques capture data on ignitable liquid components over the same volatility range. Ignitable liquids of each ASTM classification were analyzed several times utilizing various analytical methods on several different instruments. The comparison of these summed ion spectra for ignitable liquid identification, ASTM classification and sub-classification was evaluated by ROC analysis.

The results indicate the summed ion spectra of complex mixtures contain enough information to make comparisons between ignitable liquids by calculating a simple similarity metric. The cluster analysis demonstrates ignitable liquids that predominately contain alkanes are similar to one another. It also indicates ignitable liquids containing alkanes are more similar to ignitable liquids with isoalkanes than ignitable liquids containing aromatics. Duplicate analyses of the ignitable liquids have an extremely high probability of being correctly identified with the correct class and sub-class assignments having a lower probability of being correct. Analyses of the same ignitable liquids with various analytical and instrumental methods had slightly lower probabilities of correct associations than those analyzed by the same analytical method and instrument. Identification of specific product types within a broader ASTM classification and sub-classification is possible because each product contains a mixture of components in unique ratios. Each component has a unique EI mass spectrum and the resulting sum of spectra is similarly unique.

The software developed at UCF can rapidly perform the comparisons between an ignitable liquid sample summed ion spectra and a database of ignitable liquid summed ion spectra, typically searching a database of 450 entries in a fraction of a second. The database search results can assist in the determination of the ASTM classification and sub-classification of an ignitable liquid sample. The method is also applicable in the discrimination of questioned and known samples of complex mixtures. The summed ion spectral comparison method with the in-house software is being evaluated by the State of Florida Bureau of Forensic Fire and Explosives Analysis. The software has been applied to assist investigators in one missing person case and has been used to analyze and discriminate between commercial explosives.

The summed ion spectral comparison with associated software provides a complementary method to the ASTM E1618 standard method typically utilized in fire debris analysis, and may find utility in many additional physical evidence comparisons.

**Ignitable Liquids, Fire Debris, GC-MS**

## A30 Forensic Analysis of Triacetone Triperoxide (TATP) for Information on the Synthetic Route and Precursor Identity

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After attending this presentation, attendees will be able to understand the success and limitations of the approach to determine the identity of the precursor material as well as the synthetic route.

This presentation will impact the forensic science community by showing that it could be possible to determine which precursor chemicals were used in a particular synthesis of TATP, or in discriminating between different batches of TATP possibly found at multiple crime scenes.

Triacetone triperoxide (TATP) is a primary high explosive that has been linked to various terrorist attacks worldwide, including the failed attack on an American Airlines flight in 2001 by the infamous "shoe bomber", and the 2005 London subway bombings. For more than twenty years TATP has been the explosive of choice among many terrorist groups because its synthesis is relatively simple, and because the precursors used in the synthesis of TATP can be readily obtained from commercial sources. For these same reasons TATP has also become alarmingly popular among "amateur teenage scientists", as is evident by the prodigious number of amateur videos uploaded to the Internet showing the preparation and detonation of homemade TATP. The proliferation of information on the Internet regarding TATP has created an ever increasing problem for homeland security and law enforcement organizations. Though Internet recipes for the synthesis of TATP are easily found, they often differ in which commercially available precursors are recommended for use as substitutes for pure laboratory grade chemicals.

The research reported here is focused on the analysis of uninitiated and initiated TATP samples for the purpose of obtaining information about the precursor chemicals used in the synthesis, as well as gaining information that might indicate the particular synthetic route used by a terrorist or criminal. Many industrial and commercial chemicals commonly used as precursors in the illicit synthesis of TATP often contain additives and contaminants which can potentially carry through the TATP synthesis. If these additives can be detected in the final product, they might be used to forensically determine which precursor chemicals were used in a particular synthesis, or in discriminating between different batches of synthesized TATP, possibly found at multiple crime scenes.

TATP was synthesized by licensed personnel using a variety of Internet recipes, and using both reagent grade and industrial and commercial grade precursors. Precursor chemicals were analyzed for additives and impurities prior to their use in TATP synthesis. The additives and impurities were identified when possible and cataloged. Synthesized samples were then analyzed for the presence of trace impurities and additives, and matched against standards. TATP samples were detonated using a BAM Fallhamer device. Optimized analytical methodologies were developed using gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry (ESI-MS), ion mobility spectrometry (IMS), and polarized light microscopy (PLM). Data and results will be presented to demonstrate successes and limitations of this approach, and the potential forensic value of the analyses will be also be discussed.

**TATP, Peroxides, Explosives**

## A31 Classification and Discrimination of Container and Vehicle Glass by Laser Induced Breakdown Spectroscopy (LIBS)

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After attending this presentation, attendees will understand the significance of forensic glass examinations using Laser Induced Breakdown Spectroscopy (LIBS) including how the data from the analysis is interpreted.

This presentation will impact the forensic community by providing a better understanding of the capabilities, advantages, and limitations of LIBS in forensic analysis of glass.

The classification and discrimination of glass evidence can be of importance in forensic investigation of several types of cases. Glass fragments collected from a crime scene such as vehicle glass from a hit and run accident or fragments from container glass resulting from a struggle are sometimes the only evidence providing information of association between a suspect and the event. In this study, LIBS is used to classify glass fragments into an end use category and discriminate between similar container and similar vehicle glass fragments.

A 266nm pulsed Nd:YAG laser was used as the excitation source to create a very small (~2 mm diameter) plasma. A fiber optic positioned to collect the light emitted from the plasma is connected to a Mechelle spectrometer (Andor Technologies) with a wavelength range between 200nm and 900nm and a ICCD camera (Andor Technologies) thus producing a high resolution (R= 5000) spectra and a large amount of spectral information in a very short time (~1sec). The emission lines collected are characteristic of the elemental composition of the sampled glass fragments removing ng quantities of the glass, making this essentially a non-destructive technique. The samples included in this work include 40 different container and 25 different vehicle glass samples. The element menu used to classify and discriminate these two types of glass is different and one of the advantages of LIBS is that all the elemental information is available for interpretation. The laser energy was kept constant at ~25mJ throughout all the experiments. The lens to sample distance (LTSD) was optimized for the best coupling resulting in the best precision of the analysis (focusing the laser ~1.7mm into the sample).

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was also used to analyze the same set of samples and the data was compared to that obtained by LIBS. LA-ICP-MS is widely used in crime labs worldwide, but the cost of the equipment, maintenance and complicated data analysis makes LIBS a more cost-effective alternative that is very fast and easy to use and interpret.

The container and vehicle glass samples are easily classified by the elemental composition as determined by LIBS. Pairwise comparisons using the LA-ICP-MS and LIBS data/results were used for the discrimination study. All the container glass samples originating from different sources were differentiated by both LA-ICP-MS and LIBS and all vehicle glass samples known to have originated from different manufacturing sources were also distinguished by both LA-ICP-MS and LIBS. The use of LIBS has proven to be a reliable, useful technique requiring almost no sample preparation and also providing a viable alternative to the more established, but more resource intensive, LA-ICP-MS and uXRF techniques for elemental analysis of glass.

**LIBS, Glass Discrimination, Glass Classification**

## A32 Elemental Analysis of Cotton Fiber Evidence using Solution ICP-MS and LA-ICP-MS

*Jose R. Almirall, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199; and Jenny Gallo, BS\*, 8921 Southwest 142nd Avenue, Apartment 411, Miami, FL 33186*

After attending this presentation, attendees will understand the principles of Laser Ablation–Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS), the importance and use of cotton fiber evidence in the field of forensic science and how elemental analysis can help distinguish cotton samples based on elemental composition.

This presentation will impact the forensic community by introducing a method for elemental analysis of cotton fibers for the purpose of increasing the discrimination between otherwise similar cotton evidence. Elemental analysis of cotton is also beneficial to customs and the USDA because it would add an additional source of information to assist in the geographic sourcing of cotton. The basis of the sourcing and differentiation is that cotton grown in different geographic regions of the United States (or the world) will have variations in trace metals due to soil nutrients, water content and type of fertilizers used.

Fibers are very common pieces of trace evidence found at a crime scene. Cotton is the most frequent type of fiber evidence encountered. This is due to the fact that a large amount of clothing is made from cotton. To date, analysis of cotton fiber evidence is limited to class characterization, color and perhaps fracture matches. Fracture matches are fairly uncommon and class/color characterization does much information for discrimination purposes.

Currently, no method for the elemental analysis of cotton for forensic use exists and the development of a method for elemental analysis of cotton could change the way fiber evidence is used in court in the future. Trace elemental content has the potential to provide additional discrimination between very similar fibers that would otherwise not be distinguished. Much of the cotton grown in the United States is exported to other countries for manufacturing and then imported back into the United States. Linking that exported cotton to certain geographical growing regions of the United States would allow verification of the source of the cotton. The USDA is also interested in elemental analysis of cotton because it would allow quality control to verify what the manufacturer reports on the label is what the clothing is actually made of.

A digestion procedure was developed and tested using solution ICP-MS analysis yielding good precision data (< 5% RSD) for most of the element menu (ie., Mg, Al, Mn, Pb, Sr, Ba, Fe). The method was then transferred to LA-ICP-MS for bulk analysis of a pellet made from a small amount (< 200 mg) cotton sample. LA-ICP-MS eliminates digestion/solution steps (reducing cost and exposure to hazardous materials), it's relatively non-destructive, uses minimal amounts of

sample (300ng is actually introduced into the instrument for every analysis) and reduces analysis time. A comparison in precision and discrimination between conventional solution ICP-MS and LA-ICP-MS is presented. Approximately 50 white cotton samples of known geographic origins have been analyzed to determine the discrimination potential by elemental analysis and these results will be presented.

**Cotton, LA-ICP-MS, Elemental Analysis**

## A33 Analysis of Smokeless Powder Components by Capillary Electrochromatography - Time-of-Flight Mass Spectrometry

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After attending this presentation, attendees will learn how the components of of commercial smokeless gunpowder can be detected and identified using capillary electrochromatography - mass spectrometry (CEC-TOF-MS).

This presentation will impact the forensic community by providing the details of a fast and robust analytical method with minimal sample preparation that avoids the sample instability and degradation that can occur with methods such as gas chromatography.

Unburned particles of smokeless powder found at a bombing scene can be analyzed to associate evidence found at the crime scene to a particular brand of powder. This identification may lead to a source of the powder and possibly generate investigative leads.

Individual standards of commonly found smokeless powder components were prepared by dissolving 1.0 mg of each standard in 1.0 ml of methylene chloride. A mixed standard was also prepared. An aliquot of each standard was put in a vial, the methylene chloride evaporated under ambient conditions, and reconstituted in acetonitrile and 5mM sodium phosphate buffer adjusted to pH 6.8. It was found that 85:15 was the optimum ratio. A 50 cm hexyl acrylate-based monolith was prepared and conditioned with the acetonitrile/buffer solution.

All standards were analyzed with an Agilent G3250AA LC/MSD TOF run in CEC mode. For each run the capillary was conditioned with buffer solution for five minutes, followed by electrokinetic injection of the sample at 30 kV for 10 seconds. The capillary was maintained at 30 kV and 5 bar pressure during the separation and TOF-MS detection.

The results proved the efficient and reproducible separation of ten compounds found in smokeless powders: diphenylamine, dimethylphthalate, diethylphthalate, dibutylphthalate, methyl centralite, ethyl centralite, 2-nitro- and 4-nitrodiphenylamine, and 2-nitroso- and 4-nitrosodiphenylamine.

The use of CEC-TOF-MS represents a promising analytical scheme for the detection and identification of smokeless powder components. It is a fast, reproducible technique for the discrimination of smokeless gunpowder that avoids the problems presented by the breakdown of thermally labile components of smokeless powder during GC-MS analysis. Further research will concentrate on post-blast analysis of both intact and burned smokeless powder components.

**Smokeless Powder, Electrophoresis, Mass Specrometry**

## A34 Cathodoluminescence (CL) Microscopy and Spectroscopy Application to Soil/Sand

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After attending this presentation, attendees will understand the principles of cathodoluminescence (CL) microscopy and spectroscopy applied to soil/sand, as well as sample preparation, mineral component identification, digital image processing, and elemental analysis.

This presentation will impact the forensic community by illustrating the key steps in practical application of the method and its integration into techniques currently used in forensic soil/sand analysis.

This poster presentation describes and demonstrates the application of cathodoluminescence (CL) microscopy and spectroscopy to the characterization of mineral components of soil/sand. Forensic geologic samples are often comprised of varying concentrations of both light and heavy minerals, as well as foraminifera, diatoms, and other organic particles, making them amenable to identification by a variety of methods. Quartzes, carbonates, and feldspars are the most abundant minerals on Earth and, as such, are usually encountered as constituents of soil/sand samples. Because these minerals are ubiquitous, they may be found in even very small amounts of trace geologic materials such as dirt smears and dust. Application of CL microscopy and spectroscopy is suitable to differentiate among common classes of minerals, such as feldspars, carbonates, zircons, and quartzes, all of which exhibit characteristic CL colors when bombarded with an electron beam. The cathodoluminescence emission is related to the presence of trace element activators, such as $Cr^{3+}$, $Mn^{2+}$, $Mn^{4+}$, $Fe^{3+}$, and rare earth elements ($REE^{2+/3+}$), such as hafnium, neodymium, dysprosium, and europium, as well as due to lattice defects within the crystal.

Additionally, within the mineral types, cathodoluminescence microscopy and spectroscopy will provide information that can discriminate among different sources of each mineral. The additional discrimination among sources of quartz, for example, would provide a useful tool for the forensic comparison of these geologic materials. Further, CL microscopy and spectroscopy, combined with traditional forensic geologic methods, may offer information for source determination by providing information about the conditions under which the mineral was formed.

Study results presented will include: (1) suitable sample preparation for processing with multiple techniques, (2) the application of CL digital image processing, and (3) particle elemental analysis, using automated SEM-EDS and micro-XRF. The focus of this study is to develop an optimized analytical scheme for processing small sample sizes with these microanalytical methods. Considerations of sample size and sequence of analyses necessary for sample manipulation, integrity and beam damage, as well as automation of processing for high sample throughput, will also be presented.

**Cathodoluminescence, Soil, Microscopy**

## A35 Ion Beam-Induced Luminescence as a Forensic Tool

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The goal of this presentation is to introduce the principles and practice of ion beam-induced luminescence with a specific focus on the spectroscopic information that can be obtained from forensic samples and the applicability of IBIL to cases of comparison, authentication, and provenance.

This presentation will impact the forensic community by demonstrating how the ion beam - induced Luminescence (IBIL) is a new technique that is applicable to a range of questions involving the forensic analysis of trace evidence. The spectroscopic information provided by IBIL can aid in comparison, authentication, and provenance examinations of forensic materials including soil, building materials, paints, and glass.

Recent studies have demonstrated the potential for cathodoluminescence (CL) to be an important forensic tool in the discrimination and potential sourcing of trace materials that luminesce. CL is the emission of visible or near visible light from a sample that has been bombarded by an electron beam. An ion beam-induced luminescence (IBIL) method that can be used to discriminate between different minerals by bombarding them with an accelerated proton or alpha beam has been developed. Since all luminescence results from the alteration of band-gap energies due to the presence of trace elements or structural defects in crystalline materials such as minerals, both IBIL and CL produce similar UV-Vis-NIR spectra. This emission is characteristic of either the geological environment of formation of the mineral or, for a synthetic luminescent material, the manufacturing process. Luminescence is observed in many materials routinely encountered as trace evidence, including soils and rocks, building materials, glass, and pigments. The variation in luminescence for a particular mineral can therefore be used to discriminate among samples from different sources or, in certain cases, provide information about the provenance of a sample.

Many of the most abundant minerals (e.g., quartz, feldspar, and carbonate minerals) are luminescent. Due to their ubiquitous nature, these mineral components have typically been underutilized for forensic discrimination. However, the variation in luminescence within a given mineral type provides additional discrimination among sources and offers the potential for improving the significance of geological evidence. Prior research has demonstrated that cold cathode CL with light microscopy provides a relatively fast method to screen soil samples through visual identification of luminescent minerals. In addition to visual observation, high-resolution spectroscopy can offer more detailed information about specific activators (defects and trace elements responsible for luminescence) in a given mineral. For example, in feldspar minerals, the chemical composition can be estimated on the basis of the $Fe^{3+}$ emission band. In heavy minerals such as zircon, monazite, and apatite, rare earth element activators, typically present at 1-500 ppm, can be identified and quantified with high resolution spectroscopy. Together, visual and spectroscopic examination of mineral components can be combined to provide a variety of information about soil and sand samples that complement more traditionally used analytical techniques.

IBIL provides another method to measure luminescent characteristics of minerals and it has two additional features: ion microprobe analysis can be used to excite single mineral grains (on the order of 10 microns) and the system can simultaneously measure luminescent signatures and perform elemental x-ray analysis. The ability to irradiate only one mineral grain at a time prevents quenching of an entire forensic sample during examination and the addition of simultaneous particle-induced x-ray emission (PIXE) spectrometry provides additional information for forensic source attribution. This presentation will provide an introduction to the principles and practice of IBIL with a specific focus on the spectroscopic information that can be obtained from geological samples and the applicability and limitations of IBIL in cases of comparison, authentication, and geographic sourcing. Spectroscopic information will be compared with prior data collected using cathodoluminescence imaging to assess the degree of additional discrimination and value to provenance determination.

**Ion Beam-Induced Luminescence, Cathodoluminescence, Mineralogy**

## A36 Determination of the Origin of Commercial Pepper Spray Residues Using Gas Chromatography-Mass Spectrometry (GC-MS) and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

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After attending this presentation, attendees will be familiar with the feasibility of techniques using GC-MS and FTIR to classify residues of commercial pepper sprays and to discriminate them from each other. Limitations of these methods and continuing studies aimed at refining them will be described.

This presentation will impact the forensic science community by revealing a potential method of classifying and discriminating a stain residue on a suspect's clothing or other surfaces. This technique could be applicable to either include or exclude a suspect in a case where a defensive spray weapon was discharged.

Studies have been conducted to explore the feasibility of using two analytical techniques—gas chromatography-mass spectrometry (GC-MS) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)—to match an extracted pepper spray residue with its can of origin. Pepper spray samples representing cans from three different manufacturers and nine distinct lots were studied. Fabric stains from the commercial pepper spray cans were prepared on white 100% cotton tee shirts. The tee shirt stains were analyzed either by GC-MS, to determine the normalized area percents of six capsaicinoids in the sample, or by ATF-FTIR, to examine their bulk organic content. The analytical data obtained from the shirts were statistically compared to the contents of each can to determine if classification and discrimination were possible. Principal component analysis of the GC-MS and ATR-FTIR data allowed good discrimination between manufacturers, fair discrimination between some lots within the same manufacturer, and poor discrimination between cans within the same lot. Details of the experimental design, data

analysis, conclusions, and implications will be presented. Ongoing studies of these techniques will also be described.

**Pepper Spray, Classification, Principal Component Analysis**

## A37 HS-SPME/GC-MS Analysis of Various Biological Specimens Using VOCs

*Maiko Kusano, BA\*, and Eladio Mendez, Florida International University, 11200 Southwest 8th Street, Miami, FL 33199; and Kenneth G. Furton, PhD, International Forensic Research Institute, Florida International University, University Park, Miami, FL 33199*

The goal of this presentation is to provide the forensic community with an evaluation of volatile organic compounds (VOCs) present in biological specimens using headspace solid - phase microextraction/gas chromatography - mass spectrometry (HS-SPME/GC-MS).

The presentation will impact the forensic community by providing education on novel detection and analysis methods for the identification of detectable VOCs from biological specimens.

Optimum extraction times for the analysis of VOCs present in different biological specimens were evaluated. Various GC methods were also investigated. This research is a part of a larger study currently being conducted which aims to correlate VOCs found in individuals across different biological specimens including blood, breath, cheek swab, and hand odor.

The human odor is made up of a variety of organic compounds such as aldehydes, alcohols, alkanes, esters, fatty acids, and ketones. Volatile organic compound analysis in biological samples such as expired air (breath), sweat, blood, and urine has been used for various applications such as toxicology, medicine, and forensics. Over the recent years interest has increased regarding the identification of VOCs for metabolic profiling or diagnostic potentials for certain diseases that are known for its association with distinct odor.

A great deal of research has focused on HS-SPME and the determination of various volatile organic compounds across different biological matrices. HS-SPME has become extremely useful in the extraction of VOCs from biological matrices, as high molecular weight compound interferences are greatly reduced, allowing for small VOCs to be extracted from the headspace of the compound. Work in this area has involved matrices such as breath, cheek swab, and blood. Recently, SPME has been used to determine VOCs present in human scent from hand and armpit odor.

Expired air was sampled in a Teflon breath sampling apparatus. Whole blood was obtained by finger stick sampling and collected onto FTA cards. Cheek swabs were collected under typical forensic evidence collection methods. Blood and cheek swab samples were immediately transferred into 10mL headspace vials following collection. SPME-GC/MS was utilized to extract, separate, and identify the volatile components from the collected biological samples. Different SPME exposure times were investigated to evaluate the optimal extraction times for each specimen. No attempt was made to control microbial interactions with the samples as it may make contributions to the overall odor profile.

Optimal extraction times for each specimen were determined by a combination of the number of human scent compounds extracted and the abundances of selected extracted VOCs. Using the same GC method as previously determined for VOC extraction and analysis of hand odor, 21 hours was found to be the optimal extraction time for VOCs from cheek swab samples. For breath odor VOCs, the optimal extraction time was found to be 15 hours.

Through this research a novel blood analysis method is introduced; this method presents various applications, both in forensic and clinical areas. Forensically, HS-SPME provides a powerful tool in the analysis of blood VOCs, as FTA cards are widely used to store DNA. The thermal stability of FTA cards make the method described non-destructive to the sample allowing for continuous analysis of blood VOCs to take place while keeping any DNA information intact. Furthermore, preliminary research indicates a correlation between blood VOCs and human scent, thereby increasing the power and utility of the method presented here.

**Volatile Organic Compounds, Biological Specimens, HS - SPME/GC - MS**

## A38 The Optimization and Evaluation of the Headspace Analysis of Head Hair Samples for the Application as a Human Scent Source

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After attending this presentation, attendees will have been presented the most efficient methodology for the headspace analysis of head hair samples with the intention of using this matrix as an alternative source of human scent.

This presentation will impact the forensic society by demonstrating how the application of this work will be in using the specific chemical profile of volatile organic compounds emanating from the headspace of these specimens as an alternative human scent source which can differentiate the person it was collected from. There is great utility of this work due to the high frequency hairs are found within crime scenes. Currently, hairs cannot be used to identify from whom it originated from, unless there is nuclear DNA present, but it can only generally characterize the type of person it derived from. However, further investigation into the chemical odor profiles found from hair could provide a new usefulness to this type of physical evidence.

A tool of increasing applicability within the field of forensic science is human scent evidence. Human scent is defined as the most abundant volatile organic compounds (VOCs) identified in the headspace above a scent sample. It is believed that the chemical composition which constitutes human odor is a physical characteristic of the person from whom it originated, thus a new form of identification. Human scent profiles have more commonly been employed when using human scent discriminating canines for the search and rescue of missing or deceased people when implementing the idea that people leave a trail of their human scent wherever they go.

Traditionally, human hand odor is used for the analytical characterization of an individual's human scent. It is obtained when a subject places a piece of sterile gauze between their hands and clasps it for a prescribed period of time, allowing the gauze to retain the VOCs present in the hand secretions. It is then collected, separated and identified in the headspace of the collected sample by using Solid Phase Microextraction- Gas Chromatography Mass Spectrometry (SPME-

GC/MS). These human scent profiles produced are reproducible and unique to the individual who provided it. However, it is of interest to the forensic community to evaluate extraction methods of additional biological sources of human scent. Thus, the present study focuses on the optimization of the headspace extraction and identification of VOCs emanating from head hairs for the use as an alternative human scent source.

Head hair is a biological sample of interest for human scent identification owing to its structure. Hair is made up of a durable protein called keratin. The crystalline keratin is extruded from the hair follicle and appears as the hair shaft that is often associated with the appearance of hair. However, the hair follicle is surrounded by sebaceous glands and apocrine glands and the scalp is also covered in eccrine sweat glands. The secretions from these three glands coat the hair and impart it with characteristic odors. Historically, hair analysis has consisted of various steps including washing, segmentation, pulverization of hair strands and traditional digestion methods which will reduce the hair into a liquid form, prior to analytical evaluation. These extraction methodologies entail a longer extraction time and an exposure of the hair to chemicals that yield to hair decomposition. The work presented here provides a novel application of Solid Phase Microextraction (SPME) for the optimal extraction of volatiles emanating from hair samples within a variable population. The study utilized SPME in combination with Gas Chromatography/Mass Spectrometry (GC/MS) for the separation and identification of the extracted hair volatiles.

Various experiments were conducted to determine the most efficient way to extract and analyze the headspace of hairs using SPME. First, it was important to determine the best way to prepare the sample, prior to analysis. There were two considerations for the type of hair used in these experiments; should the hair closest to the scalp be analyzed or should the whole hair strand be used for the characterization of volatiles present in hair. Second, it was important to deduce the most efficient way to extract the headspace from the collection vial. Thus, conditions such as sample equilibration time and headspace extraction time were determined to ensure that the compounds present within the sample were collected and detected. Present within this study are the results for the most efficient method for sample preparation, headspace extraction and analysis of hair samples using SPME-GC/MS.

**Hair Analysis, Solid-Phase Microextraction (SPME), Gas Chromatography/Mass Spectrometry (GC/MS)**

## A39    Size Doesn't Matter... Or Does It?  The Implications of Sampling Strategies in Forensic Drug Analysis

*Niamh NicDaeid, PhD\*, Centre for Forensic Science, Department of Pure and Applied Chemistry, Royal College, University of Strathclyde, Glasgow, Scotland G11XW, UNITED KINGDOM*

After attending this presentation, attendees will have an understanding of the issues relating to sampling strategies for the analysis of illicit drugs.

This presentation will impact forensic drug community by illustrating the statistical viability or otherwise of  sampling strategies used in the analysis of illicit drug seizures.

Determination of an appropriate sample size has long been an issue with regard to the sampling of both large and small volumes of homogeneous material. It is inefficient and unnecessary for each and every unit within a population of relatively similar items to be analyzed; however, it is important that the sample size chosen results in an accurate reflection of the overall population.

Various strategies have long been used to determine appropriate sample sizes, and have been employed in many areas of forensic science, from the analysis of glass fragments to the analysis of units which may or may not contain controlled substances.

This work used and compared four commonly employed sampling strategies in industry. These were one arbitrary method, two frequentist approaches, and a Bayesian approach. Sample sizes were calculated using the European Network of Forensic Science Institutes (ENFSI) drug working group protocol for sampling. They were applied to the sampling of illegal drugs, where a large population of amphetamine tablets (n=100) was to be analyzed. Initially portions of all one hundred units were extracted and analyzed. Following this a randomly selected sub group of the larger group were re analysed where the number was determined by the sampling strategy employed.  Statistical tests were used in order to determine how well the resulting sample sizes truly represented the characteristics of the whole population.   The results of the statistical analysis are presented in this work.

**Drug Seizures, Sampling Strategy, Satistics**

## A40    Separation and Identification of Methamphetamine Enantiomers Via N - (trifluoroacetyl)prolyl chloride (TPC) and (S)-(+)-a-Methoxy-a-(trifluoromethyl) phenylacetic acid (MTP) derivatization

*Elzbieta J. Kubicz, PhD\*, State Crime Laboratory, 316 West 22nd Street, Cheyenne, WY 82002*

After attending this presentation, attendees will gain knowledge of the different methamphetamine synthesis methods, different enantiomer ratio depending on synthetic routes, and methamphetamine derivatization using chiral reagents.

The presentation will impact the forensic community by understanding changes in methamphetamine enantiomer ratio due to enforcement regulation resulting in changes of precursors and synthetic methods.

Methamphetamine has historically been considered the main "drug of choice" in Wyoming.

Recent federal and state regulations restricting easy access to precursors for the methamphetamine synthesis and the placing of synthesis reagents on the controlled list in Wyoming state statutes has helped drop the amount of methamphetamine cases by 30%. Nevertheless the problem is still of great concern.

Since the endogenous sources of methamphetamine are limited, smuggling this drug from different geographical locations is the most popular resource for supply.

Three neuralgic transportation routes: I-80 from San Francisco to Chicago, I-90 from Seattle to Boston and I-25 intersecting those two beginning at El Paso and connecting I-90 at Buffalo WY supply the majority of methamphetamine.

Although Wyoming Statutes regulate the unlawful possession of "any isomer of methamphetamine", our laboratory needed to establish a method for identifying the enantiomeric composition of seized methamphetamine samples.

Since the reduction of l-ephedrine or d-pseudoephedrine yields the enantiomerically pure d-methamphetamine, and the reductive amination of phenylacetone yields racemic d, l-methamphetamine, the enantiomer

ratio is relevant because it reflects the species of precursors and reagents used for the synthesis, the origin and synthetic method.

Usually, enantiomeric ratio of methamphetamine is measured using GC/MS method. Methamphetamine is converted to diastereoisomers with chiral-derivatization reagents and separated by gas chromatography with a nonchiral column.

The best known derivatization reagents in literature: N-(trifluoroacetyl)prolyl chloride (TPC) and (S)-(+)-α-Methoxy -α-(trifluoromethyl)phenylacetic acid MTPA were used. The enantiomeric enrichment with D-tartaric acid and subsequent derivatization with BSTFA with TMCS before analysis will be tried as well. The convenience, simplicity and speed of the different methods will be compared.

**Methamphetamine, Enantiomer Ratio, Chiral Derivatization**

## A41 Cocaine Contamination of Paper Currency in Birmingham, Alabama

*Jeremy Felix\*, and Elizabeth A. Gardner, PhD, University of Alabama at Birmingham, Department of Justice Sciences, UBOB 210, 1530 3rd Avenue South, Birmingham, AL 35294-4562*

After attending this presentation, attendes will have learned about analyte loss through different extraction methods. In addition, the methods can also be adapted and applied in any classroom setting to teach drug chemistry, techniques for extractions and GS/MS usage, and practical hands-on experience with techniques applied in forensic laboratories. The long term aim of this project is to develop purification methods for profiling impurities and precursor materials.

This presentation will impact the forensic community by adding Birmingham, AL to the major U.S. cities that have been tested for the presence of cocaine in the local currency. In addition, the researcher will be able to analyze the pros and cons for dry extraction versus acid/base extraction methods, allowing analysts in forensic labs to choose between detection limit and injecting unknown impurities in sensitive lab equipment which may result in carry over to subsequent runs.

The objective of this project was to test $1 bills in Birmingham, AL for trace amounts of cocaine and to compare both purification of the sample and sample loss on a qualitative basis using a dry extraction and an acid base extraction for both cocaine on $1 bills and levmethamfetamine (l-meth) in nasal decongestive inhalers. The attendee will learn about analyte loss through different extraction methods. In addition, the methods can also be adapted and applied in any classroom setting to teach drug chemistry, techniques for extractions and GS/MS usage, and practical hands-on experience with techniques applied in forensic laboratories. The long term aim of this project is to develop purification methods for profiling impurities and precursor materials.

There have been numerous reports of the percentage of paper currency that is contaminated with cocaine and other controlled substances in both the U.S. and internationally. However, the term "dirty money," covers more than just the presence of a controlled substance. Contamination found on currency includes nicotine, bug repellent, sunscreen, Ritalin, procaine, plasticizers, cosmetics, glycerol, and other substances (JOEL). Potentially pathogenic bacteria were found on 94% of $1 dollar bills tested in west Ohio (Pope et al. 2002) and germs of fecal, respiratory, and skin origin were found on bills from Chicago, New York City, and Washington, DC (Turner 2001).

Twenty $1 bills randomly retrieved from a Wachovia bank in the Birmingham AL area were extracted with 10 ml of methanol. The methanol extract was divided into two equal portions and the methanol evaporated. One portion was then analyzed by dry extraction into $CHCl_3$ and the other portion underwent an acid base extraction. Results from GC/MS analysis of the extractions indicate that 80% of the bills were positive for cocaine when analyzed with the dry extraction, however, the chromatogram had many impurities. Some of the impurities carried over into the blank injected between each sample. The number of bills testing positive for cocaine was much less using the acid base extraction, however, the chromatograms were cleaner and there was no carryover.

Similar extraction experiments were performed using nasal decongestion Inhalers to compare the results of dry extraction versus acid/base extraction for l-meth. Similar results were obtained: there was loss of the l-meth after acid/base extraction relative to the dry extraction. The levels of some impurities were decreased relative to the l-meth. There were some impurities that were not affected by the acid/base extraction and may, in fact, have even been enhanced. Carryover was not a problem in either of the extraction methods. This work is ongoing.

In conclusion, two methods were used to extract cocaine and l-meth from $1 bills and Vicks decongestive inhalers, respectively. For both sets of date, the acid/base extraction results in a cleaner GC/MS spectrum, is better to run on the GC column, but yields less intensity in chromatogram peaks due to sample loss through the multi-step cleaning process. Carryover of the impurities was seen in blank injections between cocaine sample runs.

**Cocaine, Currency, Extraction**

## A42 Optimization of Solid Phase Micro Extraction - Gas Chromatography/Nitrogen Phosphorous Detector (SPME - GC/NPD) for the Detection of Methyl Centralite and Ethyl Centralite From Gun Shot Residues

*Jorn Chi Chung Yu, PhD, and Brittney C. Gonzalez\*, Sam Houston State University, 1003 Bowers Boulevard, Huntsville, TX 77340*

After attending this presentation, attendees will have had the opportunity to discuss a method for detecting small amounts of methyl centralite and ethyl centralite with a novel extraction scheme of using solid phase micro extraction. The ease of adaptation of this technique to forensic labs from other chemistry - focused areas will be shown. Discussion of similar efforts towards advances in science being applied to forensics will be encouraged.

This presentation will impact the forensic community by explaining the many compounds that are specific to gun powder primers and stabilizers. For the purposes of uniqueness, methyl centralite and ethyl centralite were reported as highly significant to GSR. Detection of trace amount of methyl centralite and ethyl centralite has been a challenging task. This investigation of a novel extraction technique has created an alternative way to detect these GSR signature molecules. This new method will have a great impact on the determination of molecular marks of those GSR samples that couldn't be easily determined by conventional analytical procedures.

Methyl centralite (MC) and ethyl centralite (EC) are two signature molecules highly associated with gunshot residues (GSR). The objective of this work was to find a sensitive analytical method for extracting and identifying trace amount of EC and MC from GSR related samples.

A sensitive extraction scheme to extract MC and EC from the samples has been successfully developed, such as gun powders, un-burnt gun powder residues collected near the target. The extraction was

achieved using a solid phase micro extraction (SPME) technique. The SPME fiber was exposed to the headspace in a 2.0-mL vial that contained the sample. The vial was also dipped in an oil bath maintained at 80 degrees Celsius during extraction. After extraction, the extracts were then desorbed in a GC injection port at 280 degree Celsius for 5 mins and splitlessly injected to a gas chromatography (GC) coupled to a nitrogen phosphorus detector (NPD) for analysis. The gun powder (or un-burnt) particle samples were removed from disassembled unfired ammunition cartridges, and the burnt particle samples were taken from gun shot residue deposits near the target areas. Organic components from only one single gun powder particle, either burnt or un-burnt, were successfully extracted and analyzed by the SPME-GC-NPD. No interference peaks were overlapped with EC peak at retention time of 16.7 mins. Unfortunately, one interference peak slightly overlapped with MC at retention time 15.8 mins. Results confirm that the new extraction procedure is capable of extracting trace amount of MC and EC, as well as many other organic components from a single gun powder particle with no derivatization. This method will offer an incredible potential to identify explosives, plasticizers, and trace amount of additives from gunshot residue (GSR) evidence for forensic applications. This new method is a highly dependable, rapid and inexpensive way of identifying GSR. The Limit of Detection can vary, but it was as small as a nanogram. Found within this work was the optimal conditions of SPME-GC-NPD for the detection of EC and MC for GSR.

**SPME, GC-NPD, MC and EC**

## A43    Analysis of Sweat Components Using Headspace Solid-Phase Microextraction (HSPME)

*Mi-Jung Choi\*, Chungnam National University, 305-764, Daejeon, KOREA; Chang Hwan Oh, PhD, Deptartment of Oriental Medical Food and Nutrition, Semyung University, Jecheon, 390-711, KOREA; and Sung-Woo Park, Chungnam National University, 305-764, Daejeon, KOREA*

The goal of this presentation is to present the research results which suggest that the analysis based on individual characteristics of human sweat emanations could provide useful information for the forensic science investigation.

This research will impact the forensic community by proving that there are individual characteristics of human sweat emanations and this could provide useful information for the forensic science investigations.

Sweating is controlled from a center in the preoptic and anterior regions of the hypothalamus where thermosensitive neurons are located. The heat regulatory function of the hypothalamus is also affected by inputs from temperature receptors in the skin. Sweat is not pure water; it always contains a small amount (0.2 - 1%) of solute. When a person moves from a cold climate to a hot climate, adaptive changes occur in their sweating mechanisms. This research is observing the forensic application of human odor by sweat and grouping and identification of Korean male. Individual sweats of humans are determined by several factors. These factor divided of regardless of diet or environmental factor (primary factor), present because of diet and environmental factors (secondary odor), influenced of outside source(i.e., lotion, soaps, perfumes etc). We evaluated the components present in human sweat by headspace solid phase microextraction (HSPME) and 2nd dimensional gas chromatography (GC×GC) - time of flight mass spectrometry

(TOFMS). We collected sweat sample from three male donors. A total of 269 compounds were identified as components of human sweat. Compound classes present in human sweat, such as carboxylic acids, alcohols, aldehydes, aliphatics/aromatics, amides/amines, esters, halides, heterocyclics, ketones, thio/thioesters/sulfonyls, oxide, sulfides, nitro compounds. Common components such as alcohols (2-decen-1-ol, 2-phenoxy ethanol, 2,7-dimethyl-1-octanol, 2-butyl-1-octanol), aldehydes (nonanal, dodecanal, octanal), aliphatics/aromatics (tetradecane, 2,6-dimethyl-heptadecane, 2,3,5,8-tetramethyldecane, nonadecane, hexatriacontane, 1,3-bis(1,1,-dimethylethyl)- benzene, biphenyl, 2,6-dimethyl-naphthalene, phenanthrene) esters (4-methyl-benzoic acid, oxalic acid) heterocyclics (2,4,6-trimethyl pyridine, dibenzofuran, N-[4-bromo-n-butyl]-2-piperidinone, 1H-indole), ketones (4,6-dimethyl-2-heptanone),2-methyl-2-undecanethiol 7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin, 2,4-bis(1,1-dimethyl ethyl)-phenol were found. Individually distinct components as 2-nonenal, 2-undecenal with age and identified the influence of outside sources (soaps, lotions, perfumes) as Lily aldehyde, 2,6-dimethyl-naphthane, etc and environmental factors(dibenzo thiophene, 9H-fluoren-9-one, fluorene, fluoranthene, 9H-Xanthene, 1H-Indene) were also found. The results suggest that the analysis based on individual characteristics of human sweat emanations could provide useful information for the forensic science investigation

**Sweat, Individual Characteristic, HSPME/TOFMS**

## A44    Characterization and Testing of Pseudo Scents and Pig Organs Used as Canine Training Aids

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After attending this presentation attendees will know more about pseudo scents.

This presentation will impact the forensic science community by helping determine the best training aids to assist canines in discovering surface and buried human remains.

The recovery of human remains is not only essential for the collection of evidence in homicide investigations but also is often required by the court to convict suspects of murder, and can help provide closure for victims' families. Canines used for human remains detection (HRD) can be trained using a variety of training aids. The FBI research unit was interested in characterizing the volatile organic compounds (VOCs) above pseudo scents and pig organs to help determine the best training aids to assist canines in discovering surface and buried human remains.

To determine which VOCs were emanating over pseudo scents, several formulations were characterized using gas chromatography mass spectrometry (GC/MS) and then compared and contrasted to other training aids. The pseudo scents were first tested using solid phase microextraction (SPME) with a laboratory GC/MS, and with direct injection into the GC/MS. The same aids were then tested with a portable GC/MS system via headspace air sampler which could be used in the field to verify canine identifications. The results from these tests were compared to known compounds produced by microbiological activity and to the VOCs measured above decomposing pig organs. A further

experiment tested the pseudo scents and decomposing pig organs for insect activity outdoors to help determine whether the samples contained VOCs likely to be found near buried or surface remains.

SPME GC/MS was able to identify 26 compounds in the pseudo scent formulations. Direct injection into the GC/MS detected an additional 11 compounds. Insect activity was observed when the pseudo scents were placed outdoors, indicating that they may contain compounds likely to be found near surface remains. Based on these results, it is possible to characterize pseudo scents and determine which training aids will be most useful in the training of canines for the recovery of surface or buried remains.

**Human Remains Detection, Gas Chromatography Mass Spectrometry, Solid Phase Microextraction**

## A45 Internal Validation of the AmpflSTR® MiniFiler™ Amplification Kit for Use in Forensic Casework

*Dana M. Pilon, BS\*, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701; Kimberly D. Fiorucci, MSFS, Mesa Police Department, Forensic Services, 130 North Robson Street, Mesa, AZ 85201; and Pamela J. Staton, PhD, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701*

The goal of this presentation is to discuss the valdiation study of the AmpflSTR® MiniFilerTM PCR amplification kit with an ABI 310 Genetic Analyzer for use in a forensic casework laboratory.

This presentation will impact the forensic community by providing the basis of the five validation studies conducted according to SWGDAM (Scientific Working Group on DNA Analysis Methods) and DAB (DNA Advisory Board) standards: Sensitivity, Concordance, Mixtures, Peak Height Ratio Analysis, and Stutter.

The AmpflSTR® MiniFiler™ PCR amplification kits utilize a five-dye fluorescent system for fragment analysis and have been optimized for the analysis of degraded and/or inhibited DNA samples. The MiniFiler™ kit was designed with miniSTRs to amplify eight of the largest sized loci in the AmpflSTR® Identifiler® PCR Amplification Kit (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA). These loci along with the gender-identification locus, Amelogenin, enables simultaneous amplification of the loci that often fail detection during the amplification of degraded DNA. An internal validation of the MiniFiler™ amplification kit was completed for the Mesa Police Department Forensic Services Section for use in casework analysis.

DNA samples used for these studies included buccal swabs, hairs, cigarette butts, NIST-traceable bloodstain samples, semen samples, and touch samples. Samples were quantitated using Applied Biosystems Quantifiler™ Human DNA Quantification Kit. All samples were amplified with both the AmpflSTR® Identifiler® and MiniFiler™ amplification kits and electrophoresed on a 310 Genetic Analyzer. The data was analyzed using GeneMapper® ID v.3.2 analysis software.

The MiniFiler™ amplification kit yields reliable and robust DNA profiles when the input DNA amount is between 0.2-0.6 ng, with the optimal template amount being approximately 0.4 ng. However, DNA results were obtained with a DNA input amount of 12.5 pg. A significant increase in pull-up and off-ladder alleles was seen at concentrations greater than 0.625ng. Experimental results obtained from the MiniFiler™

amplification kit were concordant with the results obtained from the Identifiler® amplification kit. In the mixture study, full profiles were detected for the minor contributor at 1:2 and 1:4 ratios with a threshold set at 50 rfu. More dilute mixtures (1:8 and above) gave some results for the minor contributor, but not consistently. The peak height ratios between the two heterozygous peaks were above the recommended values from ABI and averaged approximately 83%. Heterozygote peak imbalance as low as 41% was observed within one loci of a given sample. Based on the empirical data obtained from the samples, the average stutter percentages fell below the manufacturer's published guidelines.

Based on the results of these studies, the use of GeneMapper® ID analysis software in conjuction with the MiniFiler™ amplification kit will yield results consistent with those currently found using GeneMapper® ID with Identifiler® amplification technology. It will also yield more complete profiles when used in combination with the Identifiler® amplification kit. This validation study supports the use of the MiniFiler™ amplification kit on forensic casework samples that are degraded, contain PCR inhibitors, and/or have low template DNA amounts.

**MiniFiler™, Validation, Degraded DNA**

## A46 PowerPlex® S5 as a Simple, Cost Effective DNA Profile Screening Tool

*Laura K. Young, BS, BA\*, 115 Dots Circle, China Grove, NC 28023; and Amy McGuckian, MSFS, and Cecelia A. Crouse, PhD, Palm Beach Sheriff's Crime Lab, 3228 Gun Club Road, West Palm Beach, FL 33406*

The goal of this presentation is to discuss the usefulness and applicability of Promega's PowerPlex® S5 mini STR system as a screening tool in forensic laboratories. The PowerPlex® S5 kit is composed of five multiplexed mini STR loci that may be used to screen multiple evidentiary stains to determine if they could have been contributed by the same or different individuals. The purpose of this evaluation is to determine if the use of the PowerPlex® S5 kit ultimately reduces the cost and labor of casework analysis and provides a significant amount of discrimination that would make its use feasible. The studies performed included sensitivity, cost effectiveness, degree of discrimination and a mixture evaluation.

This presentation will impact the forensic community by providing initial evaluation data for PowerPlex® S5 so that labs may have a better understanding of the system's advantages and disadvantages prior to purchasing it for their lab. PowerPlex® S5 is designed to decrease the cost and labor required for potentially extraneous casework analysis. By screening evidentiary stains with the S5 miniplex, there will be a decrease in the number of samples that require full analysis. The costs of materials, labor and time should be dramatically decreased as well. Conversely, this will increase the throughput for forensic labs and ultimately help to decrease the number of backlogged cases.

The PowerPlex® S5 loci include D8S1179, D18S51, FGA, TH01 and Amelogenin. The largest amplicon is FGA and it is less than 260bp. Of the 18 known samples used in this study, concordance was found between the DNA typing results obtained from PowerPlex® S5 and PowerPlex® 16 kits on all samples. All of the samples were analyzed using an Applied Biosystems 3130xl with GeneMapper®ID software v3.2. Each sample

was injected at 3kV for 3, 5, and 10 seconds. The 5 second injection results are discussed here as that is Promega's recommendation for sample injection time.

Sensitivity studies included 5ng, 2.5ng, 1.26ng, 0.625ng, 0.312ng, 0.156ng, 0.078ng and 0.039ng. A full S5 profile was generated for 5ng through 78pg Allelic dropout was detected at 39pg. The same sensitivity samples were analyzed with PowerPlex® 16 and it was found that full profiles were obtained at 5ng through 156pg and dropout occurred at 78pg DNA. However, the optimal amount of DNA for PowerPlex® S5 was found to be between 0.2 and 0.6ng. This makes PowerPlex® S5 an ideal system for use in forensic casework labs where evidentiary DNA concentrations are not always abundant.

PowerPlex® S5 employs hot-start DNA technology. The *Taq* DNA polymerase is included as part of the system. This eliminates the need to purchase *Taq* separately and helps to decrease the cost. Based on reagents only, the cost per reaction with PowerPlex® S5 is $10/reaction compared to $17.50/reaction with PowerPlex® 16. If PowerPlex® S5 was employed as a screening tool and minimized the number of samples that required analysis with PowerPlex® 16, the cost savings could be dramatic. Also, because fewer samples are being analyzed with PowerPlex® 16, the analysis time will be shortened. The five loci of PowerPlex® S5 require much less time to analyze and samples can be quickly screened as evidentiary relevant or not.

Within the samples analyzed in this study, 14 were from the same family. This was done to demonstrate the power of discrimination of PowerPlex® S5. All 14 familial profiles were different. The power of discrimination of these 14 samples ranged from 1 in 36,000 to 1 in 1.7 million within the Caucasian database at the Palm Beach County Sheriff's Office. With this high level of discrimination, PowerPlex® S5 is a powerful tool in screening samples from mass disasters or in reducing the number of samples that require conventional STR analysis..

Mixed DNA samples were evaluated based on the percent of total loci where a mixture was indicated. For a 1:1 female to male mixture, PowerPlex® 16 indicated a mixture at 63% of the loci. As the female to male mixture increased, the ability of PowerPlex® 16 to discern a mixture drastically decreased. However, PowerPlex® S5 indicated a mixture at 40% of the loci for a 25:1 female to male mixture.

The data obtained in this evaluation indicates that PowerPlex® S5 would be a cost effective and reliable screening tool for forensic casework labs.

**PowerPlex®, Mini STR, DNA**

# A47 Streamlining and Optimizing the Procedures of a State Databank Lab

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After attending this presentation, attendees will gain an understanding of some of the challenges faced by databank laboratories, as well as possible alternative solutions to these problems. Attendees will also be shown examples of how one such laboratory is implementing these solutions to produce quality data with a minimum of expense.

This presentation will impact the forensic community by highlighting economical automated procedures and potential optimization studies that can be conducted.

Kansas legislature recently passed a law requiring DNA collection on all felony arrestees. With this influx, the requirement for sample processing methods that produce high quality data, and are also efficient and cost effective is imperative. The Kansas Bureau of Investigation (KBI) has chosen to investigate possible implementation of several procedures to try to meet this need. The number of backlogged samples can be reduced by employing high pH extraction, fluorometric quantitation, multiplex amplification, and expert system analysis for databank samples. These procedures will also decrease profiling costs significantly and increase analyst efficiency. To ensure that each protocol was fully optimized before validation, the extraction, quantitation, and amplification procedures were evaluated in preparation for the introduction of an expert system.

The streamlined process begins with the Bode DNA collectors for buccal samples, a Bode modified BSD-600 Duet punching instrument, and KOALA (Kansas Offender Arrestee Laboratory Application). KOALA is custom software which tracks sample barcodes and creates input plate maps for the BSD; this process increases punching efficiency and performs several quality control checks. Optimization experiments were designed to demonstrate consistent punches in the correct orientation, with no sample carry-over after cleaning strikes.

Next, extraction and quantitation are performed with the Biomek® 2000 liquid handler. The high pH extraction is a simple and inexpensive technique[1] that lyses buccal cells to release nuclear DNA into solution.[2] This method utilizes heat, sodium hydroxide, and tris-hydrochloride. After the DNA is extracted, Quant-iT™ OliGreen® ssDNA reagent is added to quantitate the samples with a fluorometer. The simplicity of these procedures allows the user to extract and quantitate DNA on the same worksurface in less than 30 minutes. Reproducibility and accuracy studies were executed to prove this method was reliable and did not introduce carry-over.

Normalization and PCR set-up are also carried out with the Biomek® 2000 and Promega's Genetic Identity Normalization Wizard with previously validated methods.[3] Using a single multiplex kit such as PowerPlex® 16 rather than their current amplification kits, Profiler Plus® and COfiler®, reduces the number of plates necessary for these steps and those further downstream. Experiments were designed to assess concordance and reproducibility with PowerPlex® 16. By combining all these automated procedures with high-throughput capillary electrophoresis on the 3130*xl* Genetic Analyzer, an analyst can take a plate of samples from punching to data collection in a single workday. Following validation and implementation of these procedures, the KBI can focus on data collection for the validation of an expert system for data review. "For forensic DNA analysis, expert systems could easily be one of the most important advances in analyzing convicted offender samples.[4]" The manual data review process is lengthy and requires a significant time commitment of analysts; an expert system will alleviate some of the burden for single source samples.

As more states pass arrestee DNA collection laws, the forensic database laboratories are challenged with increasing sample throughput in a cost effective manner. If this challenge is successfully addressed, CODIS will continue to be an outstanding judicial system resource. Automated procedures can provide an economical solution, although optimization and validation are required. This presentation gives an example of one state databank laboratory's quest for cost effective

methods that increase efficiency and data quality. It illustrates possible solutions to the challenges the forensic community faces.

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**Automation, Fluorometer, High pH Extraction**

## A48    The Benefit of Using the Qiagen MinElute® PCR Purification Kit for Post PCR Cleanup on Low Level DNA Samples

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After attending this presentation, attendees will be informed of the benefits and ease of implementing Post PCR clean up to boost their DNA signal on low level samples.

This presentation will impact the forensic community by demonstrating that there are simple and cost effective methods to implement into the laboratory system that can improve results from low level DNA samples.

This study was conducted to demonstrate the benefits of including the Qiagen MinElute® PCR Purification Kit into the analysis scheme on forensic samples containing low quantities of DNA.

The study was designed to evaluate the similarities and differences in capillary electrophoresis signal detection when using the Qiagen MinElute® PCR Purification Kit on amplified DNA obtained from commonly used short tandem repeat (STR) commercial amplification kits. The STR amplification methods used in this study were the Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, Identifiler® kit, Minifiler™ kit, and the Yfiler® kit, and Promega's PowerPlex® 16 system, PowerPlex® Y system, and the PowerPlex® S5 system.

The Qiagen MinElute® PCR Purification Kit uses a silica membrane to bind DNA fragments ranging in size from 70 bp to 4 kb. While the DNA is bound to the membrane, impurities such as unwanted primers, salts, enzymes, unincorporated nucleotides, dyes, oils, and detergents flow through the column. Removal of these impurities ensures that more DNA is injected during the electrokinetic injection on the instrumentation, thus increasing the fluorescent signal intensity.

All single source samples were extracted using a standard organic extraction method and quantitated using the Applied Biosystems Quantifiler® Human DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System. Serial dilutions were prepared from DNA

extracts at the following concentrations: 1.0, 0.5, 0.25, 0.125n, 0.0625, 0.03125, 0.015625, and 0.0078 ng and amplified with each STR multiplex following the manufacturer's specifications using an Applied Biosystems GeneAmp® PCR 9700 thermal cycler. A portion of the amplified DNA from these dilutions was purified using the Qiagen MinElute® PCR Purification Kit. Both purified and non-purified samples from each of the dilutions were separated and detected using the Applied Biosystems 3130*xl* Genetic Analyzer. The data were analyzed using GeneMapper® ID Software v3.2 using a threshold of 75 rfu. The fluorescent signals from both purified and non-purified samples were compared for all eight STR multiplexes to assess the change in fluorescent signal, stutter ratio, heterozygosity, and baseline noise.

Preliminary results of low level samples have shown increased signal levels after clean up using the Qiagen MinElute® PCR Purification Kit. This has consistently been shown during preliminary trials using Applied Biosystems' AmpfℓSTR® Profiler Plus® kit, Cofiler® kit, and Identifiler® kit. Results for the dilution series using all of the above listed amplification kits will be presented in the poster.

Crime laboratories have seen an increase in the submission of requests for analysis on evidentiary items with low quantities of DNA. This study demonstrated that the Qiagen MinElute® PCR Purification Kit consistently increased the fluorescent signal with the eight evaluated commercial STR amplification kits. This purification kit can be integrated into the laboratory process with little effort for method validation and at minimal cost. Integration of the Qiagen MinElute® PCR Purification Kit into the DNA analysis procedure is a simple, cost effective method that can be easily implemented by a crime laboratory to increase the overall sensitivity of their DNA analysis methods.

**Qiagen, Post PCR, Low Level DNA**

## A49    Evaluation of the Scent Transfer Unit (STU-100) for the Non - Contact Sampling of Volatile Compounds

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This goal of this presentation is to educate the public on the use of the Scent Transfer Unit for the collection of volatile compounds.

This presentation will impact the forensic community by enhancing public understanding of the Scent Transfer Unit.

The study of human scent has been of interest to the forensic science community due to its application to human scent canines used for scent trailing and scent identification line-ups. Human scent as forensic evidence is based on the concept that each individual has a unique scent profile, similar to a fingerprint or DNA profile. Today nearly all police departments use canines to locate, based on scent, missing people or suspects in one form or another. Improving the scientific understanding of human scent components, as well as, improving the collection and delivery of such components will expand the understanding of human scent collection in the forensic community and enhance the admissibility of such evidence in the courts.

Human scent can be collected by direct contact sampling, in which a piece of material or an object is placed in direct contact with the subject of interest, or by non-contact sampling with dynamic airflow devices such as the STU-100. The Scent Transfer Unit™ or STU-100 is currently used

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by law enforcement for the non-contact sampling of human scent volatiles. With this device a scent pad, usually a piece of gauze is placed at the head of the device, and air is pulled through the device, extracting volatiles onto the scent pad to be collected.

For validation and optimization of the STU, standard volatile compounds that are commonly found in human scent were evaluated. One representative compound from each of the following functional groups was chosen for analysis: alcohols, aldehydes, aliphatics, ketones, and fatty acid methyl esters. Controlled odor delivery devices were created, by spiking the compound(s) of interest onto an absorbent material and placing it into a polymer bag. Using gravimetric analysis, the rate of dissipation was determined for the selected compounds through several types of polymer bags, including low density polyethylene and high density polypropylene bags. The appropriate permeable bag was then selected for each compound in such a way to minimize the differences in dissipation rates of the compounds.

In order to reduce background contamination during scent collection, a human scent collection chamber was designed. The chamber was fabricated in such a way that positive pressure could used to remove human scent compounds from the chamber by allowing air from outside of the chamber to flow in through a filter removing a majority of the unwanted compounds. As air flows through the filter into the chamber, the polluted air remaining in the chamber is pushed out through openings in the front wall of the chamber, thus creating a clean environment for sampling.

Following the preparation of the controlled odor delivery devices, samples were collected inside the collection chamber using the STU. After collection with the STU, the samples were placed into vials and allowed to equilibrate. The headspace of the vial containing the scent pad was analyzed using SPME/GC/MS. The variables that were studied for optimization included the flow rate of the STU, material composition, and geometry of the absorber.

While the STU-100 is currently used by law enforcement in this country, few studies have been conducted regarding the evaluation of variables associated with sample collection. In order to enhance collection of scent from human subjects, it is beneficial to evaluate the STU-100 in a controlled environment utilizing an array of standard compounds commonly reported to be components of human scent so that trends can be accurately determined and reported.

**Human Scent, Scent Transfer Unit, Volatile Collection**

## A50 Internal Validation of AmpfℓSTR® Minifiler™ Amplification Kit for Metropolitan Police Department

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After attending this presentation, attendees will understand the requirements and principles behind an internal validation for a start-up forensic laboratory.

This presentation will affect the forensic community by providing DNA requirements and recommendations relative to the validation for Metropolitan Police Department of the AmpfℓSTR® Minifiler™ amplification kit that other forensic laboratories may utilize in their own validation efforts.

The AmpfℓSTR® Minifiler™ kit was designed to reliably type degraded, inhibited, low-copy number nuclear DNA samples. Samples with low copy number or inhibition may now generate full or partial profiles which were previously unable to be genotyped. This amplification kit is able to perform this function by generating smaller polymerase chain reaction products for eight loci. These loci include D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA and Amelogenin. Internal validation is a required process for every forensic laboratory before a new kit or instrument can be used for casework. The goal of this project was to validate the Minifiler™ amplification kit for Metropolitan Police Department for use in DNA identification while also utilizing VALID™ software. The VALID™ software provides detailed steps and suggestions to take for completion of this project. The tests performed included a precision, sensitivity, accuracy and reproducibility and a mixture study. Samples originally were analyzed at 40 rfu and results from the individual tests required that this number be increased. Throughout the course of the validation, while using pristine DNA samples, there were a significant number of artifacts seen. These included significant off-ladder alleles, -A and secondary stutter which is recognized by Applied Biosystems at the D13 locus. After the completion of the tests required by VALID™ a study was performed to try and type samples that showed poor genotyping results using the Identifiler™ amplification kit. The results from this study, eight samples with target concentrations of 0.025 and 0.05 ng/ul, were 83 new alleles that were previously not typed using Identifiler™. This validation demonstrated excellent concordance with the previous alleles typed using Identifiler™ and demonstrates that the amount of DNA required for reliable typing may be a range between 0.025 ng/ul and 0.05 ng/ul with an RFU threshold of 85 to 100 rfu.

**Validation, Minifiler™, DNA**

## A51 Application of Forensic Palynology in Two Murder Cases

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The goal of this presentation is to show people that are not aware of forensic palynoly how this forensic science can be used and how it works.

This presentation will impact the forensic community by demonstrating a type of forensic science (forensic palynology) that has been neglected in most forensic institutions but in many cases can help to solve some crimes.

In this presentation a computerized semi-automatic 3D forensic cranio facial reconstruction tool is demonstrated. This forensic application is based on a large scale database of facial soft tissue depths of Caucasian adults and a flexible statistical model of face shape used in computerized three dimensional (3D) craniofacial approximations.

This presentation will impact the forensic community by implementing a large scale database of facial soft tissue depths into a 3D forensic cranio facial reconstruction tool which allows for specific correction of gender, age, and body posture.

Mass communication of forensic facial reconstruction models in unsolved identification cases can stimulate recognition by relatives and may provide records to accomplish further comparative analysis. The majority of the reconstruction techniques use earlier published facial soft tissue depth charts collected on cadavers or in vivo. Traditional 3D facial reconstruction techniques apply modeling clay or Play-Doh on a cast of the skull, approximating the estimated tissue depths at the landmarks and interpolating in between. Recent different computerized techniques are evolved to obtain more objective 3D facial soft tissue estimations. In this presentation the application of the implementation of soft tissue thickness described by De Greef et al (2006) into a flexible statistical model of face shape developed by Claes et al (2006) is demonstrated.

De Greef et al performed in vivo facial soft tissue depth measurements on 967 adult Caucasoids employing a user-friendly, fast, mobile and well validated ultrasound measuring device. Data of both sexes, varying in age and body mass index (BMI) were collected at 52 manually indicated facial landmarks. The "A-scan" industrial ultrasound device was selected to perform the tissue dept measurements because of its low weight, compactness, facile transport and its ability to connect a 6mm diameter, 10MHz ultrasound transducer which can easily be pointed to the landmarks during analysis. The repeatability of the ultrasound measurements was tested on a subset of 33 volunteers and their accuracy proved after comparing the ultrasound measurements and the soft tissue thickness calculated from total head CT-scans on 12 patients.

The computer-based combined flexible statistical model for craniofacial reconstruction established by Claes et al requires the achievement of a skin surface and tissue depths database, a statistical face and soft-tissue depth model and a statistical model fitting procedure. The skin surface shape of approximately 350 individuals were captured with a mobile 3D photographic device, after measuring thickness and marking the 52 soft tissue landmarks and registering age, gender and BMI of all the individuals. The constructed statistical facial surface and soft tissue depth tissue model consists of a geometrically averaged facial template together with a correlation-ranked set of modes of principal variations or face-specific deformations that capture the major changes or differences between facial outlooks and their skull-based landmarks in the database. The created elastic mask is subsequently fitted to the external surface of the individual craniofacial skeleton such that all the 52 landmarks of the mask fit the corresponding target skull-landmarks and the estimate of the nose tip.

Multiple reconstructions of the same skull but with different combinations of age, gender and BMI can be made within a few seconds. More specific facial soft tissue changes during aging can be simulated. The automatic adjustment or improvement of the model using face specific modes of variation, results in unbiased and more realistic 3D facial soft tissue reconstructions.

Forensic palynology deals with the application of pollen and spores in solving legal issues, either civil or criminal. The main forensic application is in determining the possibility of associative evidence. Here, two murder cases are shown where forensic palynology helped to link people and objects to the crime scene. In one case, the pollen assemblages from the victim's hair and shoes were compared with soil collected from several places in the crime scene. A very high similarity was observed between the pollen assemblages from the victim's hair and from the soil collected in one of the places sampled from the crime scene. This indicated that the victim fell in that particular spot and was not moved from there. In the other case, pollen assemblages from the suspect's belongings and the victim's hair and shoes, were compared with vegetation (*Adenocarpus complicatus* and *Dittrichia viscosa*) and the

pollen assemblage from soil collected in the crime scene. The suspect's shirt and the victim's hair had many *Dittrichia* and *Adenocarpus* pollen (>90%) indicating that they have been in contact with the bushes from the crime scene. The pollen assemblages from the victim's shoes and the soil showed a high similarity with each other. These results very strongly supported the contention that the suspect had been at the crime scene and that the victim was not moved from the place where she was found.

**Palynology, Murder, Investigation**

## A52    Optimization of FSS-i³ for Future Use at the Michigan State Police CODIS Unit

*Jacqueline M. Jarzombek, BS\*, Marshall University-Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will know about the i-STRess component of the FSS-i³ software system and the process of optimizing such Expert System to mimic the guidelines of the Michigan State Police's CODIS Unit.

This pressentation will impact the forensic science community by helping reduce backlogs caused by data review with the help of Expert Systems, such as FSS-i³.

With the implementation of automation, convicted offender DNA samples are currently collected, extracted, and amplified substantially faster than they can be manually reviewed. To reduce the data review backlogs, software programs, known as Expert Systems, are currently available for forensic laboratories to facilitate data interpretation. Using National Institute of Justice funding through the FY 2006 DNA Capacity Enhancement Program (2006-DW-BX-K122), the CODIS Unit of the Michigan State Police recently purchased the FSS-i³ Expert System with the intent of using it as an additional tool for data analysis.

The FSS-i³ software was created by the Forensic Science Service® in the United Kingdom and is licensed for retail sales in the United States through the Promega Corporation. FSS-i³ needs the assistance of another software system, such as the Applied Biosystems GeneScan® and Genotyper® or GeneMapper® *ID,* for the purpose of calculating size, height, and area of allelic peaks. The FSS-i³ has 3 components: i-STRess, i-STReam, and i-ntegrity, which allow single source samples, two person mixtures, and possible contamination to be reviewed. The primary focus of this project is the evaluation of FSS-i³ for the analysis of single source samples using the i-STRess component.

In order for the Michigan State Police CODIS Unit to utilize the FSS-i³ Expert System, the software must undergo a validation study to demonstrate its ability to perform at the same level or higher than a human analyst. The software must be validated using data generated with the laboratory's instrumentation and amplification kit. For this study, data was generated on an Applied Biosystems (ABI) 3100 Genetic Analyzer with ABI Data Collection Software 1.1 and ABI's GeneMapper® *ID* Software version 3.2 was used to organize height and area of each allele before introducing the data to the FSS-i³ software. In the i-STRess application, the laboratory must optimize the rules and settings to mimic their specific data interpretation guidelines. The criteria for this determination will be that the software system will either pass samples with correct allele calls or flag problematic samples that show evidence of artifacts. A human analyst will then decide whether the flagged sample can be passed or failed. Over 1,500 known samples were processed using the i-STRess component of the FSS-i³ Expert System to optimize the rules and settings to reflect the Michigan State Police CODIS Unit's

interpretational guidelines. Using the FBI Laboratory's guidelines for validations on Expert Systems, a calibration set of at least 200 known samples exhibiting artifacts or explainable abnormal electrophoretic migration of DNA fragments was used to test the final rule settings. Once the testing of the software is completed, it will be sent to the National DNA Index System Custodian for validation approval.

**Expert System, FSS-i³, Optimization**

## A53 Characterization of Y-STR Loci in a Population From Nicaragua (Central America) and Study of Population Substructure

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After attending this presentation, attendees will have been provided with information on the Nicaraguan genetic population data for the most used forensic genetic markers of Y-chromosome and on population substructure.

This presentation will impact the forensic community by providing Y-chromosome genetic data with a great potential for forensic applications in the Nicaraguan population and by assessing if the population substructure affects forensic statistical calculations.

Population genetic databases are of utmost importance in forensic genetics. However there are still some important population groups not enough characterized genetically. This is the case of certain Central American populations as those from Nicaragua. There is a lack of information on Nicaraguan population from a genetic point of view and this can be an issue when solving criminal, paternity and identification problems. On the other hand, it is important to determine the degree of substructure within the population and the effects that it may exert on the forensic statistical calculations.

In this survey, a population of healthy Mestizo male individuals from Nicaragua (Central America) were typed for 16 Y-STR markers and tested for the degree of substructure within them. For sampling, blood drops from 147 healthy unrelated donors born and living in Nicaragua were collected on FTA cards. DNA extraction and Quantification: Genomic DNA from the blood stains was extracted using Chelex® 100 (Sigma, Germany), and the quantity of human DNA was determined by real time PCR using the Quantifiler Human DNA Quantification kit (Applied Biosystems, Inc.). PCR and typing were carried out by using the Ampfℓ STR® Yfiler kit (Applied Biosystems, Inc.). Amplification was performed in a GeneAmp® Thermal Cycler 2720, and typing was carried out in an ABI Prism 310. Fragment size and allele designation of different loci was determined by comparison with allellic ladders

provided with the kit and analysed using the GeneMapper ID v3.2.1 software (Applied Biosystems, Inc.).

The recommendations of the DNA Commission of the International Society of Forensic Genetics for analysis of STRs systems were followed. Gene diversity at each locus, the number of haplotypes and haplotype diversity were calculated using a program developed by Chakraborty and Lee (http://cgi.uc.edu/download/haplo). Linkage disequilibria tests, with 5000 permutations, were performed using the same program. The $F_{ST}$ values were calculated according to Weir and Cockerham (1984). The analyses performed on the 16 Y-chromosome loci studied in the present study have a great potential for forensic applications in this population.

**Y-Chromosome, DNA Typing, Population Substructure**

## A54 The NIJ DNA Property Crimes Demonstration Program: The LAPD Experience

*Gregory B. Matheson, BS\*, Los Angeles Police Department Criminalistics Laboratory, 1800 Paseo Rancho Castilla, Los Angeles, CA 90032*

After attending this presentation, attendees will have a general understanding of the NIJ DNA Property Crimes Demonstration Program and a specific understanding of both the pitfalls and successes experienced by the Los Angeles Police Department, a participant in the Program.

This presentation will impact the forensic community by providing information laboratories can use to evaluate the value of utilizing DNA technology to investigate property crimes within their jurisdiction.

Five sites participated in a demonstration program sponsored by the National Institute of justice. The purpose of the program was to study if it makes economical sense to utilize DNA technology for the investigation of property crimes. The study design required each site to collect biological evidence from five hundred scenes of property crimes. Of the crimes, 250 would utilize DNA technology as an additional investigative tool. The remaining scenes would be processed utilizing standard investigative tools. Following the conclusion of the collection phase of the program, the test group and control group were compared. Major points for evaluation were solve rates, conviction rates, sentencing, and cost for investigative and prosecutorial processes for each solved crime.

This presentation will focus on the experiences of the Los Angeles Police Department (LAPD) as one of the demonstration sites. From grant application, through labor relation issues to analysis and hit rate, the LAPD acquired considerable information and experience that will be used to influence future allocation of existing biological evidence and investigative services and impact requests for future increases in biological analysis resources. With a significant backlog of requests for the analysis of DNA in violent crimes, utilizing limited DNA technology resources on property crimes does not, on the surface, appear prudent. However, participation in the program has shown that in Los Angeles, the hit rate between burglary crime scene evidence and individuals in the CODIS offender database has proven to be higher than in any other crime category. In addition, the criminal histories of the identified offenders were usually significantly more serious than the burglary for which they were arrested.

The presentation is offered to share the more significant finding of

the LAPD experience to others considering the value of incorporating the utilization of biological analysis in the investigation of property crimes.

**DNA, Burglary, Grant**

## A55 Recovery of Contact DNA from Handled Handguns

*Mounir Moudouni, MS\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Forensic Biology Laboratory, Houston, TX 77054*

After attending this presentation, attendees will gain knowledge of the locations on handguns where DNA is most likely to accumulate and therefore most conducive to maximizing the chances of generating a DNA profile. The number of alleles detected from a controlled study of five different areas of three popular handguns, namely the grip, the slide, the magazine lips, the safety/slide release, and the trigger are presented. Post-PCR purification of low yield samples to enhance the quality of the fluorescence signal is also addressed.

This presentation will impact the forensic community by demonstrating the areas most likely to produce detectable amounts of human DNA material during the collection of biological evidence from firearm evidence such as handguns. The information in this study will also help law enforcement and DNA examiners address the question of whether it is better to first send a firearm for DNA analysis or fingerprinting. The increased chances of DNA recovery associated with preserving such evidence and the precautions that must be taken when handling handguns found at crime scenes will assist criminal investigations especially in unsolved cases. Deciphering the genetic profile associated with handled areas on a handgun may link a suspect to a crime scene and provide valuable information to criminal cases.

Varying success rates have been reported for the retrieval of contact DNA from handled objects. It is of interest to investigate and to understand factors that improve the recovery of DNA from handguns. In this study, three commonly recovered handguns, a Smith & Wesson 9mm, a Sig Sauer® 9 mm, and a High Point 40 SW-B were subjected to a controlled study of DNA recovery following limited handling. Five different areas were swabbed from each gun: the grip, the slide, the safety/slide release, the trigger, and the magazine lips. Negative and positive controls were also collected from each handgun.

The guns were swabbed prior to any testing as a positive control. To collect negative control samples, the handguns were first cleaned with 10% bleach followed by 95% ethanol and then swabbed. Subsequently, the handguns were handled for two minutes by a single person, who loaded each gun and fired 3 rounds prior to collecting test swabs. DNA extraction, quantitation and amplification were performed using a low-yield Chelex procedure, the Quantifiler® Human DNA Quantification Kit, and the Ampfl STR Profiler Plus DNA Typing Kit. Capillary electrophoresis was prepared by mixing 1µL of amplified product with 9µL of Rox size standard - formamide mixture followed by injection of the samples for 10 seconds on an ABI 3100-Avant.

The resulting profiles showed that the grip, the slide, and the safety/slide release represent the best areas for collection of DNA material. The 9mm Smith & Wesson produced the highest number of alleles when the grip of the gun was swabbed, most likely due to its rubberized texture, while the Sig Sauer® and the High Point were characterized by a high number of alleles at the slide and the safety/slide release areas, respectively. Surprisingly, the trigger did not yield positive test results for two of the three guns. These results suggest that the best

areas for collecting DNA from a handgun will vary depending on its design and operation. Peaks were observed in negative control samples from two of these guns. This observation suggests that some surfaces in these guns are difficult to clean, even with bleach, suggesting that it is possible to obtain DNA from handguns even if they have been cleaned.

Post-PCR purification of the amplified products using a modified Microcon-100 procedure with an elution volume of 25µL and 3µL of injected product led to a two to three fold increase in the number of alleles detected from the test samples. This technique allows visible "under threshold" peaks to be resolved above threshold level. The value and limitations of post-PCR purification for recovery of low yield samples will also be discussed.

**Contact DNA, Handguns, Post-PCR Purification**

## A56 The Effect of a Latent Print Processing Technique on the Recovery of DNA From Duct Tape

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The goal of this presentation is to educate attendees on the ability to separate duct tape with liquid nitrogen, process the tape for latent prints, and still recover evidential DNA.

This presentation will impact the forensic community by demonstrating the effective recovery of DNA from chemically treated duct tape evidence.

The purpose of this study was to determine if a separation and latent print processing technique would adversely affect the recovery of DNA from duct tape.

Latent prints and DNA are two critical pieces of evidence recovered from duct tape. Finding both latent prints and DNA on duct tape would be powerful evidence in linking a suspect to a scene or victim. Performing latent print processing using a wet suspension of fingerprint powder on duct tape prior to DNA sampling could potentially destroy DNA. This study concentrated on a single separation and latent print processing technique and whether DNA profiles are still obtainable post-processing.

A single roll of commercially available duct tape was used for all samples in this study. Three test subjects were chosen to handle the duct tape in order to deposit both latent prints and DNA. For the first test, both the backing and adhesive sides of the tape were handled. For the second test, only the adhesive side of the tape was handled and for the third test, the duct tape was simply grasped and torn. The tape was then cut into three sections for treatment. One sample was an untreated control, the second was for separation, and the third was for latent print processing.

The separation process uses liquid nitrogen in order to facilitate the removal of duct tape from itself, as well as other objects. Previous work has shown that liquid nitrogen facilitates the separation of duct tape and will not alter latent prints. Using liquid nitrogen on duct tape momentarily deactivates the adhesive and allows separation without excessive pulling and stretching of the tape. Any stretching could potentially distort any latent prints present.

Developing the latent prints was the second part of the analysis. There are numerous latent print processing techniques, some of which are used primarily for adhesives. The technique used for this study is a

commercially available fingerprint powder suspended in a liquid surfactant. This suspension was brushed onto both the backing and adhesive sides of the duct tape and then rinsed with deionized water. After rinsing with water, the remaining powder adheres to any latent prints left behind. Once samples were treated with liquid nitrogen or processed for latent prints, they were packaged and sent for DNA analysis.

DNA analysis of the duct tape consisted of an extraction procedure involving the addition of Proteinase K, followed by a clean-up and concentration procedure. Real-time PCR was used for quantitation, followed by amplification consisting of 28 cycles, and detection using a genetic analyzer.

The DNA results obtained from the tape samples were compared to each of the test subjects' DNA profiles. A positive human quantitation result was obtained from all samples. Variation in the number of loci detected was observed in the control samples as well as samples processed with liquid nitrogen or fingerprint powder.

**DNA, Duct Tape, Latent Print Processing**

## A57 The Analysis of Various Types of Cotton and Polyester as Swabbing Mediums for Low Copy Number DNA Recovery

*Christina M. Mulligan, BS\*, and Lawrence Quarino, PhD, Cedar Crest College, 100 College Avenue, Allentown, PA 18104*

After attending this presentation, attendees will understand how to optimize low copy number (touch) DNA recovery from smooth, non-porous substrates by determining the most effective swabbing medium.

This presentation will impact the forensic community by proposing a more efficient swabbing medium than the commonly used cotton swab to prevent loss of DNA during the collection process.

Previous research performed by the authors suggested that a cotton swab was not optimal in swabbing low copy number DNA samples. Furthermore, this research demonstrated that cotton and polyester in the form of swatches have a preferential ability to remove DNA from glass substrates as compared to the other fabrics tested in the study. These fabrics included nylon, wool, acrylic, and a polyester swab. This previous research also utilized an extraction protocol for samples with lower concentrations of DNA.

This study focused on four different studies that deal with testing different types of polyester and cotton for DNA recovery. Three of the studies utilized ten different types of cotton and ten types of polyester to determine the solvent conditions that are most conducive to the highest DNA recovery possible. Saliva was used as a source of epithelial cells to determine which characteristics of the fabrics attributed to the ability or inability of the fabric to recover DNA from a smooth, non-porous substrate. For each of these studies, thread count, fabric weave, orientation of the weave, electrostatic charge, polarity of the solvent and molecular properties were noted. Smaller quantities of DNA that are more representative of low copy number DNA samples were utilized to mimic case work samples.

Case work samples were mimicked by moistening the twenty fabrics with sterile water and swabbing a neat and 1:100 dilution of human saliva on a glass substrate to compare DNA recovery. A second part of this study focuses on the influence of electrostatic charge on each of the cotton and polyester fabrics. This study was carried out the same as the moistened fabric study using the same saliva sample, except that the

fabrics were not moistened with any solvent. A third study focused on employing a solvent with a different polarity to moisten the fabric to determine if the polarity of the solvent influences DNA recovery using the same saliva sample.

The fabric samples were extracted using a low copy number DNA extraction and quantified using a human-specific *Alu*-based real time quantitative PCR assay. Raw quantitation values in ng/uL were obtained for each of the samples. Statistical analysis was used to determine if there was a significant difference between the concentration of DNA recovered for the fabrics comparing different solvents.

The most effective combinations of fabric and solvent determined from the first three studies was used to perform a fourth study utilizing touch DNA. The samples collected for this study were fingerprints on glass surfaces, mimicking case work touch DNA samples.

Results of the study show that cotton woven fabrics with a low thread count have a preferential ability to recover low concentrations of DNA. Molecular interactions between the solvents, the cell membrane, and the fabrics confirm these results and can aid analysts in choosing effective fabric swabbing mediums for the recovery of low copy number and touch DNA evidence.

**LCN DNA, Fabrics, Swabbing Mediums**

## A58 Comparison of Surfactants for the Transfer of Touch DNA

*Lyndsie N. Schantz, BS\*, 700 Forbes Avenue, Apartment 1816, Pittsburgh, PA 15219*

After attending this presentation, attendees will have learned about the effectiveness of different surfactants employed for the collection of touch evidence from held objects for downstream extraction of DNA.

This presentation will impact the forensic community by indicating which surfactant yields the highest concentration of DNA thereby resulting in a higher probability of obtaining a complete genetic profile.

Attendees of this presentation will learn about the effectiveness of different surfactants employed for the collection of touch evidence from held objects for downstream extraction of DNA. Three objects commonly processed by forensic crime labs: firearm grips, hats and spent bullet casings have been swabbed with two experimental surfactants along with a water control using the double swabbing technique.

A surfactant is a solution that can greatly reduce the surface tension of water. Touch DNA is the transfer of DNA molecules to a solid surface of an item via the deposit of cells through the handling of that item. Using the traditional double swabbing technique, low copy number (LCN) DNA may be lost because of the high water content of fingerprints and subsequently touch DNA. Using a surfactant allows a protective coating to be formed around the transferred material and the hydrophilic ends associate with the water from the touch DNA. The traditional double swab technique uses water as the surfactant which could further dilute the LCN DNA. It is believed that the use of ethanol and sodium dodecyl sulfate (SDS) as a surfactant retains a greater amount of DNA. Studies have shown that ethanol, an alcohol used at even a concentration of 20% reduces the surface tension of water. SDS is a detergent which contains hydrophilic properties allowing greater transfer of the compromised DNA left by touching an object.

Both male and female volunteers were recruited for this study, for a total sample size (N) of 20. Prior to swabbing, the nonporous objects were wiped with a 100% ethanol solution, air dried, and exposed to

ultraviolet light to reduce contamination from previous handling of the object. Each subject was asked to handle the item in a way that would replicate everyday use. The objects were swabbed using the double swab technique. The initial swab was moistened with either a 1:1 absolute ethanol and Nanopure Water solution or 100% SDS solution, followed by a dry swab to collect any extraneous solution left behind. The process was repeated for each surfactant and the control with a one week interval between each experimental condition. An interval of one week allowed for the regeneration of surface epithelial cells. On average it takes thirty days for a skin cell to go from the most inner part of the epidermis to the outer layer. For the purpose of this study complete regeneration is not necessary therefore a one week interval between experimental conditions is more than sufficient. The top of the swabs were cut and stored in a 1% PBS solution before the extraction process. Both the dry and wet swabs were combined in the extraction process using the commercially available Qiagen QIAamp DNA Blood Mini Kit. Extracted DNA was quantified via real-time PCR. A multiplex of mini-STR primers was used in the PCR reaction. Genotyping was performed on an ABI 3100-Avant Genetic Analyzer. Preliminarily results support the 100% SDS as being the most effective surfactant.

**Surfactant, Touch DNA, Double Swabbing Technique**

## A59 The Persistence of Foreign DNA Under Fingernails

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After attending this presentation, attendees will have a better understanding of the background levels of foreign DNA that may be present under fingernails and the impact it has on the evidentiary value of DNA collected from fingernail debris. They will also learn of the collection technique that is most efficient for isolating foreign DNA that may be present under the nail.

This presentation will impact the forensic community by examining the background levels of exogenous DNA present under an individual's fingernails, and how it may be important in clarifying the value of DNA evidence recovered from fingernail debris. Comparing different techniques used to collect fingernail evidence will make it possible to implement the technique that produces the most complete profiles from exogenous DNA while minimizing the endogenous DNA profile into evidence collection performed by investigators, sexual assault nurse examiners, and pathologists.

In January 2005, a Michigan college professor was murdered; foreign DNA found under her fingernails did not match the primary suspect, who was later convicted of criminal sexual conduct and murder. A confession by another individual and further investigation resulted in the initial conviction being overturned. This lead to the question, how common is it for foreign DNA to be present under an individual's fingernails?

Throughout an investigation, police seek to connect a suspect to the scene or the victim through evidence or witness statements. DNA analysis of debris collected from under a suspect or victim's fingernails

has been used to link a suspect to a victim as the fingernail provides an isolated and somewhat protected area for DNA-containing cells to accumulate (Cook and Dixon, 2007). In a violent struggle, as might occur during a sexual assault or murder, the victim may attempt to defend themselves against their attacker. During this process, the victim's nails may come into contact with the perpetrator, allowing for the transfer of biological material between the victim and suspect.

In contrast, activities such rubbing the face or eyes, scratching, or biting fingernails may increase the concentration of the donor's own cells under the nails. If foreign DNA is present, these activities will cause a mixture to be observed, increasing the difficulty of interpreting the resulting DNA profiles. Swabbing and scraping are techniques often employed for the collection of fingernail evidence, although these have not been optimized to alleviate the problem of mixtures. Cline *et. al.* (2003) developed a type of differential extraction to isolate the exogenous DNA found on a nail from the endogenous DNA found in the nail. The technique involved soaking nail clippings in a solution that effectively isolated the exogenous DNA that was present while leaving the nail intact. The DNA in solution could then be carried through the analysis process. Since the nail remains undisturbed, the likelihood of detecting a mixture due to the degradation of the nail itself is minimized.

Prior contact between a victim and suspect is sometimes used to explain the presence of the suspect's DNA under a victim's fingernails. However, Cook and Dixon (2007) swabbed nails from 100 individuals and examined them for mixtures. It was demonstrated that only a small amount of exogenous DNA was found under the fingernails and the only significant ($p < 0.05$) results for mixture profiles were obtained from males who had been in physical contact with another individual in the 24 hours prior to sample collection. This indicates that when a strong mixed profile is observed from a fingernail sample it is not likely due to previous casual contact between the victim and the suspect.

The goal of the current research was to compare different techniques, specifically cutting, scraping, and swabbing, to determine which is the most effective at obtaining foreign DNA that may be present under a nail while limiting the risk of contamination from the nail itself. A second goal was to clarify the evidential value of fingernail evidence by examining the background levels of foreign DNA under an individual's fingernails.

Study participants were asked to superficially scratch another participant and different techniques were utilized to collect any cells under the nails. Further, the undersides of fingernails of random individuals were swabbed. DNA extractions were performed, followed by STR analysis and comparison to control (buccal) profiles. The various techniques tested showed differences in their ability to limit the amount of nail DNA carried through the analysis. Also, a relatively small percentage of individuals carried foreign DNA under their nails if they had not been in purposeful physical contact with another person.

**DNA, Fingernails, Swabbing**

## A60 DNA Analysis of Biological Material on the Adhesive Surface of Electrical Tape Recovered From Post - Blast Pipe Bomb Fragments

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After attending this presentation, attendees will understand the two main objectives of this work. First, the results of a comparison of multiple methods to extract DNA from the adhesive side of electrical tape will be presented. The most efficient method was then used to successfully extract and analyze DNA from the adhesive surface of electrical tape from post-blast pipe bomb fragments.

This presentation will impact the forensic community by discussing another DNA analysis tool for identifying the manufacturer of a pipe bomb by analyzing the post-blast fragments.

The purpose of this study is to investigate the feasibility of analyzing DNA recovered from biological material deposited on the adhesive surface of electrical tape used in the construction of a pipe bomb. Previous research has demonstrated the ability to obtain DNA profiles from the outer surfaces of end cap and pipe nipple fragments from post-blast pipe bombs. Another material commonly used when constructing pipe bombs is electrical tape. Oftentimes, nails or other shrapnel may be taped to the outside of the device or the tape may be used to hold the fuse or wires in place. Biological material deposited on the adhesive surface at each end of the tape as the tape is applied to the device could directly link the assembler of the device to the device post-blast. The chance that the biological material recovered from the adhesive surface of the tape was deposited by a person unrelated to the construction of the device or by multiple individuals is relatively small. Furthermore, the biological material is protected from the products of combustion and other potential environmental insults that may be encountered during and after deflagration of the device.

This study was divided into two parts. The first part of this study investigated various methods to recover and purify the DNA from biological material deposited on the adhesive surface of the electrical tape. Ten different methods were examined and compared. These methods included the use of different types of swabs, solvents, and various extraction methods. The most efficient method involved the use of a foam swab moistened with an adhesive remover solvent to collect the biological material followed by an organic extraction using a centrifugal filtration device to concentrate the DNA.

The next step in the study was to test the effectiveness of the selected method on post-blast fragments. Six pipe bombs were constructed with PVC components and four pipe bombs were constructed with galvanized steel components. Aliquots of a cell suspension were dried overnight on the adhesive surface of electrical tape. The tape was then wrapped around each of the devices. Aliquots of the cell suspension were also placed in marked areas on the exposed pipe nipple and allowed to dry. A separate PVC and steel device were similarly prepared to be used as controls. All of the devices, except the controls, were then deflagrated in a controlled manner and the fragments were recovered. Three of the PVC devices and one of the steel devices were highly fragmented and the tape recovered was not amenable to further analysis.

At the laboratory, the cell spots on the adhesive surface of the electrical tape were collected using a foam swab moistened with an adhesive remover solvent and extracted following an organic extraction method. The DNA was concentrated, as necessary, using a centrifugal filtration device. The cell spots on the exposed surface of the pipe nipple were collected using a cotton swab moistened with deionized water followed by a dry swab. The swabs were then extracted using a commercially available DNA extraction kit. The concentrations of human DNA in the extracts were determined using real-time PCR. STR amplification was performed using a commercially available multiplex amplification kit and the fragment separation/detection was achieved using capillary electrophoresis.

Two main conclusions can be drawn from this study. The use of the foam swab moistened with an adhesive remover solvent to collect the biological material followed by an organic extraction is an effective method of recovery of DNA from the adhesive surface of electrical tape. Second, a significantly greater quantity of DNA can be recovered from the adhesive surface of the electrical tape compared to biological material deposited on the exposed surface of the pipe bomb.

**DNA, Pipe Bomb, Electrical Tape**

## A61 DNA Analysis of Improvised Explosive Devices That Employ Wireless Electronic Mechanisms for Detonation

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After attending this presentation, attendees will become familiar with the utility of analyzing electronic triggering mechanisms that may be used with improvised explosive devices (IEDs) as a means of determining the identity of the assembler.

This presentation will impact the forensic community by providing an alternative method for the investigation of IED attacks by focusing on the triggering mechanism rather than analyzing the explosive device itself.

In recent years IEDs have been used both domestically and internationally for unconventional warfare and terrorism. The means by which an IED is constructed and utilized in an attack can vary extensively. Some of the more advanced IEDs use a wireless triggering mechanism typically composed of a cell phone, two way radio, or other small electronic device that can receive a signal from great distances. Components including a touch tone circuit board, a power supply, and electrical wire are also needed to transfer the signal from the triggering mechanism to the detonator.

In past research our laboratory has examined the feasibility of obtaining a genetic profile directly from an IED following handling and detonation. Due to the poor state of DNA in shed skin cells, along with extreme temperatures of the deflagration, only highly degraded DNA is generally recovered from the resultant bomb fragments, decreasing the chance of obtaining a genetic profile of the assembler. In addition, the IED often fragments into small pieces making it difficult to collect a sufficient amount of material for DNA analysis. Focusing on the triggering device instead of the IED itself may result in increased potential for obtaining a complete genetic profile, for a variety of reasons. First, an electronic triggering device utilizes multiple components that require assembly, thus increased handling, resulting in a greater

accumulation of touch DNA. Second, DNA on the triggering mechanism may not experience the same heat levels as the IED, and therefore resist degradation. Third, the triggering mechanism can separate into its individual components (wireless device, battery, circuit board, etc.), instead of fragmenting into many small pieces as does an IED, making its recovery much easier. Finally, depending on how the triggering mechanism is attached to the detonator of the IED, there can be substantial separation or obstacles between the two, resulting in decreased damage during the blast.

In the research to be presented, participants were asked to mock assemble an electronic triggering mechanism consisting of a plywood base, clamp for a pipe bomb, cell phone or two way radio, battery, circuit board, and wire. They were also asked to handle either a steel or PVC pipe (with end caps) that was used for the explosive. Pipes were filled with smokeless powder and affixed to the mock triggering mechanism. The pipe bombs were then detonated by fuse in a controlled environment, after which pieces of the mechanism were collected. DNA was removed from the individual components using a double swab technique. Following an organic extraction, the DNA was quantified and analyzed using miniSTRs. Reference samples from the volunteers were also analyzed and assignments were made blind. Preliminary results indicate the success rate of identifying an individual who handled the triggering mechanism is as high or higher than identification from IED fragments.

**DNA, Improvised Explosive Device, Triggering Mechanism**

## A62    Internal Ballistics: Temperature Analysis of a 9mm Firearm With Thermal Imaging Camera

*Philip S. Nase, BS*, 131 South 20th Street, Pittsburgh, PA 15203; and Daniel Watsula, BS, Ronald Freeman, BA, and Lisa Ludvico, PhD, Duquesne University 341 Fisher Hall, 600 Forbes Avenue, Pittsburgh, PA 15282*

After attending this presentation, attendees will learn about the internal ballistics of a Glock 9mm semi-automatic handgun. More specifically, they will learn about the thermal environment inside of the chamber immediately following discharge of the weapon.

This presentation will impact the forensic community by presenting a novel experiment to accurately study the internal ballistics of a semi-automatic firearm and determine the temperature to which a shell casing and DNA are exposed to during firing. The data that is acquired will also be used to study the likelihood of genotyping DNA that is exposed to similar temperatures as those in the chamber.

Attendees of this presentation will learn about the internal ballistics of a Glock 9mm semi-automatic handgun. More specifically, they will learn about the thermal environment inside of the chamber immediately following discharge of the weapon. Recently there have been multiple studies, including a companion study to this experiment, which is attempting to transfer, extract, and genotype DNA from spent shell casings. It has previously been thought that the heat of firing destroys DNA rendering it useless for analysis. This study analyzes the thermal environment to which the touch DNA on bullet shell casings is exposed to upon firing. After extensive research and communication with multiple ammunition manufacturers and law enforcement agencies, it was determined that the temperature that a shell casing is exposed to during firing is not available or published.

This presentation will impact the forensic community by presenting a novel experiment to accurately study the internal ballistics of a semi-automatic firearm and determine the temperature to which a shell casing and DNA are exposed to during firing. The data that is acquired will also be used to study the likelihood of genotyping DNA that is exposed to similar temperatures as those in the chamber.

A study was performed using five different brands of ammunition, each 115 grain 9mm Luger. Each brand was tested using a 9mm Glock semi-automatic handgun. Temperatures were taken of the chamber, with the slide of the gun locked back, both before and immediately following firing using an Evolution 5200 thermal imaging camera (MSA manufacturer). Temperature readings were recorded for each brand of ammunition after single firings and multiple burst firings to observe the transfer of heat to the metal components of the firearm, mainly the chamber. Two thermal imagers were used at the same time to read the temperatures so that an average and standard deviation between the two instruments could be determined. Also, Heat Sensor Labels by Ladder Technologies were cut out and adhered to the bullet cartridge. Upon firing, the heat sensor indicates if the metal was exposed to temperatures in excess of 300 degrees Fahrenheit. The results of the heat sensor tests will be compared to the thermal imager data to discuss the amount of time it takes for the heat inside the chamber area to dissipate, and the accuracy of this experiment.

Upon obtaining significant temperature data, a study will be performed to observe the effects of the temperatures inside of the chamber on control DNA samples. Samples, 1 to 5µL in size containing epithelial cells of known concentration suspended in PBS will be pipetted onto a metallic surface that is set to the temperature recorded in the chamber area of a fired weapon. The samples will be immediately swabbed off of the surface using a double swabbing technique, extracted, quantified, and genotyped using a 5 STR miniplex developed for a companion study to observe the likelihood of obtaining DNA from surfaces that have been exposed to high temperatures for only a fraction of a second.

**Internal Ballistics, Firing Temperature, Touch DNA**

## A63    Extraction, Quantification, and Analysis of DNA From Spent Shell Casings

*Daniel Watsula, BS*, Philip S. Nase, BS, Ronald Freeman, BA, and Lisa Ludvico, PhD, Duquesne University, 341 Fisher Hall, 600 Forbes Avenue, Pittsburgh, PA 15282*

After attending this presentation, attendees will be educated about obtaining DNA profiles from the most commonly used spent bullet casings. Attendees will also learn about a modified mini - STR plex that will be used to identify the bullet casing DNA against a reference sample.

This presentation will impact the forensic community by: (1) demonstrating that DNA is not destroyed in the firing process (as commonly perceived), (2) examining the loading order of the bullets in relation to the deposition of touch DNA on the bullet casings, and (3) further establishing the utility of the mini - STR panels in forensic casework.

According to the FBI[1] in 2006, 29.3% of all murders, robberies, and aggravated assaults were committed with a firearm. Usually, when detectives arrive on the scene, the only evidence related to the gun used is spent shell casings. Frequently, spent shell casings or unfired rounds are sent to crime laboratories for fingerprinting with limited, if any successful results. While loading the magazine of a handgun, DNA is deposited on the bullet via the shedding of the epithelial cells. Several studies involving Transfer DNA have shown that a held object can readily

yield Low Copy Number (LCN) DNA. Thus, the forceful contact required to load a handgun magazine can easily lead to the shedding of epithelial cells and LCN DNA.

It has previously been thought that the heat generated by the firing of a handgun degraded or destroyed the LCN DNA left on a shell casing. In a companion study, the temperatures inside the chamber of a gun of which the shell casing and DNA are exposed were analyzed. Past research has shown that it is possible to recover partial and full profiles from spent shell casings using a miniplex.[2] The research to be presented will utilize a miniplex developed at Duquesne University to aid in LCN DNA research. The loci used include D8S1179, D16S539, D5S818, D3S1358, and amelogenin.

Subjects involved in the present study were asked to load a Glock 9mm magazine with ten bullets. After loading, each subject fired the entire magazine under the control of former Pittsburgh Police Major Crimes Unit Commander Ronald Freeman. The shell casing was collected post ejection via autoclaved wooden toothpicks and placed in a paper bag. Each bullet casing was assigned a random number by an individual not involved in the study, thus creating a study blind. Reference samples were taken at a later date, via buccal samples and were similarly assigned a random number. At no point in the study were the research subjects directly identified or linked to their coded identification number.

The experimental design involved ten study subjects, seven males and three females. The skewed sex ratio was determined based on the number of handgun crimes committed by each sex according to the most recent crime statistics for the City of Pittsburgh based on charges for VUFA (Violation of the Uniform Firearms Act). Of these 301 charges that occurred during the time period between 1/08 and 6/08, 96% of the violations were committed by males. The most commonly used handgun was a 9mm, which is consistent with the national rankings (per.comm. Ronald Freeman). Locally, of the 301 charges of VUFA, 28% were for 9mm handguns. Similarly, the use of Federal brand ammunition in this study was determined with the assistance from Pittsburgh Mobile Crime totaling up to 21% of the recovered casings.

DNA from the individual shell casings were transferred using a modified double swabbing technique that used a 50% ethanol solution as a surfactant. The swabbing tops were cut and placed in a 1X PBS solution. DNA was extracted using a modified buccal swab protocol from the commercially available Qiagen QIAamp DNA Blood Mini Kit. Samples were quantified via real-time PCR. Multiplex PCR was performed on the samples and utilized approximately 100ng of DNA. Genotyping was performed on an ABI 3100-Avant Genetic Analyzer. Preliminarily results support all three aims identified in this study.

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**LCN DNA, miniSTRs, Shell Casings**

## A64    Lateral Thinking in Crime Scene Investigation

*H. Dale Nute, PhD\*, Florida State University at Panama City, 4750 Collegiate Drive, Panama City, FL 32405; and Niamh NicDaeid, PhD, Centre for Forensic Science, Department of Pure and Applied Chemistry, Royal College, University of Strathclyde, Glasgow, Scotland G11XW, UNITED KINGDOM*

After attending this presentation, attendees will understand the nature of lateral thinking and its application to crime scene investigation.

This presentation will impact the forensic community by improving the investigative techniques of the crime scene community.

All forensic science examinations require logical thinking, creative thinking, and judgment. The latter two, however, are required at a much higher degree in crime scene investigation than in the usual crime laboratory examinations. Typically, judgment in crime scene investigation requires assessing alternate theories of the crime. Generation and development of these theories requires creative thinking. Once generated, the case theories are eliminated or confirmed using logical reasoning and empirical testing via the scientific method. The problem is these plausible theories have to be generated during emotionally draining investigations and within restricted time periods. Affirmative techniques for generating theories are needed rather than leaving that aspect to chance. A technique proposed for generating creative ideas called lateral thinking has been incorporated in some sectors of the forensic science community but is largely unknown in the majority.

The term lateral thinking was coined in 1970 by Edward De Bono to describe an approach to overcome some of the tendencies of the mind towards confirmation bias. Similar terms for the approach are "thinking outside the box," "generating alternative theories" and "contrary thinking." The value in the approach is not in its definition, however, but in its application. Published techniques for improving lateral thinking, however, are often in the abstract, obviously do not include crime scene investigation examples, and, in many cases, are irrelevant or seem to be inappropriate. Many of these mechanisms are techniques commonly used by crime scene investigators albeit with different names. And, some of the inappropriate seeming techniques may actually be of value.

The basic principle of crime scene investigation is that of evidential reasoning. The difficulty with drawing a conclusion via evidential reasoning is usually blamed on a lack of information. However, sometimes the real difficulty may be that another idea is in the way. Focusing on the origin of the bullet may cause one to miss the value of the fact it has a flat, smooth nose and thus is a ricochet. A third difficulty is that a psychological block develops when a solution is too smooth and clear. The homicide staged as a "locked door" suicide is an example in which the generation of alternative theories may be especially difficult because there seems to be no need for an additional theory if the criminal has done his job well.

Notwithstanding that logic has been taught for centuries, most crime scene investigators still have trouble applying it in a particular case and especially have trouble explaining their reasoning. Consequently, they subconsciously allow confirmation bias to occur and resist changing their minds during the investigation. The value of multiple theories is that one begins with the plausible solutions and one is more likely to keep an open mind until the conclusion. Having methodically eliminated all plausible alternative theories but one, the investigator can better defend the surviving conclusion. The technique of creating and using a theory

testing chart exemplifies a straightforward, methodical example of the use of lateral thinking that is useful in not only conducting an investigation but also in explaining one's conclusions.

Illustrative cases are presented as examples in which lateral thinking proved to be of value.

**Creative Thinking, Judgment, Investigation**

## A65 Examination of a 13-Year-Old Crime Scene for a War Crimes Trial or "It Is Too Late to Examine the Crime Scene"

*Peter J. Diaczuk, BS, 445 West 59th Street, New York, NY 10019; and Thomas Kubic, PhD\*, 8 Pine Hill Court, Northport, NY 11768*

After attending this presentation, attendees will have heard a brief history of the event, the theories of both sides of the trial, and how the physical evidence supported one side and refuted the other.

This presentation will impact the forensic community by showing how the use of the scientific method and critical thinking overcame the potential difficulties of assessing a thirteen-year-old crime scene.

Most of us are at least somewhat familiar with the basic facts concerning the genocide, other war crimes and crimes against humanity that took place in Rwanda in the 1990s. The PBS documentary *Ghosts of Rwanda*, the movie *Hotel Rwanda* and Lt. General Roméo Dalaire's book, *Shake Hands with the Devil: The Failure of Humanity in Rwanda*, have all brought the horrific events of Rwanda's 1994 genocide to mainstream attention. There have been a number of arrests, prosecutions, convictions, and punishments of individuals associated with these events. Less publicized was the April 1994 murder of ten Belgian military personnel who were assigned to the United Nations peace-keeping force (UNAMIR). The ten soldiers had been directed to assist in the protection of the then Prime Minister Agathe Uwilingiyimana, who was subsequently assassinated.

Forensic evidence regarding the killing of the ten Belgian soldiers, presented at the United Nation**s**' International Criminal Tribunal for Rwanda**,** Trial Chamber II, in June 2008, will be presented and discussed. This evidence involved the examination of the scene of the slaying of the Belgians at Camp Kigali, as well as the residence of the Prime Minister, where she was shot to death. The crime scene examinations and shooting reconstructions took place in the Rwandan city of Kigali in January 2008. The analyses revealed evidence that was critical to the defense of four Rwandan military officers who were charged with complicity in these crimes. The prosecution alleged their complicity from the accounts of "eye-witnesses". The prosecutor claimed that the highly trained and disciplined troops under the officers' command were present at both sites with crew-served heavy weapons and armored vehicles. The four officers were alleged to have participated in the shooting at the Prime Minister's Residence and at a building where the Belgians took refuge in Camp Kigali, where the shooting consisted not only of small arms but also of mounted machine gun, cannon, and heavy mortar fire. The prosecution claimed that the defendants' guilt arose from the fact that they ordered the actions, and if not so ordered, then they at least knew or should have known the events were taking place and the four did nothing to stop the murderous activities of the troops under their command.

The most critical portions of the crime scene at Camp Kigali had been preserved as a memorial to the Belgians and as a Genocide Museum, making the examination of the scene possible after so many years.

Conclusions reached by the mere careful examination of the Camp Kigali scene were confirmed by the recovery of physical evidence. Some limited off-site simulations further strengthened these opinions.

Eventually, the witness' accounts of who was firing what, from where, at the Kigali scene were found to be inaccurate. Similar review of the witness' testimony regarding events and activities at the Prime Minister's residence revealed that they were similarly incredulous.

This presentation will discuss both the theory of the prosecution, which was mainly based on eye-witness testimony, and our findings and the basis of our opinions, which are in conflict with the foregoing theory. The approach, methods employed, and the results obtained from the examination of the thirteen year-old crime scene will be discussed. Ultimately, the statement that "It is too late to examine the crime scene" was proven to be erroneous and much indeed was learned from an "old scene" by employing the scientific method.

**Rwanda, Shooting Reconstruction, Scientific Method**

## A66 FORESIGHT Update: A Business - Metric Study of Forensic Laboratories

*Max M. Houck, MA\*, 1600 University Avenue, 208 Oglebay Hall, West Virginia University, Morgantown, WV 26506-6217*

After attending this presentation, attendees will have been presented the study model and metrics for the evaluation of 17 forensic science laboratories in their operations in a business setting.

This presentation will impact the forensic community by providing a study model for any operational forensic laboratory that wishes to improve its quality, efficiency, or costs.

FORESIGHT is a business-guided self-evaluation of forensic science laboratories across North America. The participating laboratories represent local, regional, state, and federal agencies. Faculty from the WVU College of Business and Economics are providing assistance and guidance. The process involves standardizing definitions for metrics to evaluate work processes, linking financial information to work tasks and functions. Laboratory managers can then assess resource allocations, efficiencies, and value of services—the mission is to measure, preserve what works, and change what does not. While the Census of Public Crime Laboratories and the International Association for Identification Forensic Service Providers Survey approach the forensic industry broadly, FORESIGHT will show processes, strategies, resources, and allocations at a highly detailed level. A project of this magnitude for forensic laboratories has not been carried out anywhere.

Seventeen laboratories, ranging from local to federal, are voluntarily participating in this study. Only those laboratories performing the top 25 percentile were identified: The goal of the study is to improve performance not punish "poor" performance. Because all the laboratories are using the same definitions and measuring all the metrics in the same way, the laboratories can use the results of the study to compare results between laboratory demographics of which are similar, such as size of jurisdiction, and services offered, a number of employees. Initial indications are that improvement should come quickly; for example, the cost per sample for a solid dose drug analysis had a range of an order of magnitude. Local economics obtain, of course, and costs such as salaries, overhead, and supplies will vary by locale but not to that degree. FORESIGHT will be useful in the short term to improve laboratories immediately but the real payoff will come as the project continues in out

years and trends begin to emerge. Unless and until forensic laboratories operate more like business as, the logjam of resources, cases, and lag time will only increase.

**FORESIGHT, Efficiency, Costs**

## A67 Implementing Sequential Unmasking Procedures

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After attending this presentation, attendees will gain an understanding of how observer effects have the potential to compromise the interpretation of a forensic analysis, and at what stage of the analysis more information should be revealed to further refine the interpretation in the context of the extant case. Attendees will understand the impact of context effects on an analysis, and will be able to construct an administrative and analytical flow that increases the objectivity of the results and interpretation of their analysis.

This presentation will impact the forensic community by enabling laboratories to implement more objective procedures in the analysis of physical evidence, benefiting the entire criminal justice system by providing more reliable information about physical evidence collected from a crime scene.

Observer effects are rooted in the universal human tendency to interpret data in a manner consistent with one's expectations. This tendency is particularly likely to distort the results of a scientific test when the underlying data are ambiguous and the scientist is exposed to domain-irrelevant information that engages emotions or desires. Despite impressions to the contrary, analysts often must resolve ambiguities, particularly when interpreting difficult evidence samples such as those that are limited, deposited on a potentially-interfering substrate, contaminated, or degraded. With advances in technology, many forensic tests are used to analyze marginal samples likely to produce ambiguous results, such as older samples, samples exposed to environmental insult, and limited samples resulting from incidental contact. Consequently, the need for measures to minimize the consequences of observer effects in forensic testing is growing.

The full potential of forensic testing can only be realized if observer effects are minimized. These problems can be minimized by preventing analysts from having information inconsequential to the proper analysis of an item, and proceeding through interpretation in a step-wise fashion, with more information revealed only after traits of the questioned item have been characterized and documented. As but one example of this concept, sequencing the laboratory workflow such that evidentiary samples are interpreted, and the interpretation fully documented, before reference samples are compared is suggested.

**Sequential Unmasking, Observer Effects, Context Effects**

## A68 Does Non-DNA Evidence Still Have a Role in Criminal Investigations?

*Vincent J. Desiderio, MS\*, New Jersey State Police Office of Forensic Sciences, NJSP Central Laboratory, 1200 Negron Road, Hamilton, NJ 08691*

After attending this presentation, attendees will have a greater appreciation for the role of non-DNA evidence in criminal investigations.

This presentation will impact the forensic community by increasing the community's awareness of the relevance of non-DNA evidence. This presentation is intended to redirect the focus away from DNA-only investigations back towards a collaborative approach to scientific investigations.

Forensic science is a broad term that encompasses numerous discreet fields. The primary goal of these collective fields is to provide information so that proper investigations into criminal and/or civil matters can be conducted. Forensic science is unique amongst the various investigative fields primarily due to the fact that it uses the scientific method to elicit facts from physical evidence. By using the scientific method, a logical approach can be utilized to develop theories on how things may have occurred and/or why things may appear as they are. As in any other scientific field, the theories that are presented in forensic science are only theories and therefore must withstand scrutiny and challenges that can, and will, occur. Only by withstanding such challenges can a theory gain strength and general acceptance.

Forensic biology, more commonly known as DNA analysis, is just one discipline amongst the forensic collective. The products of the scientific method, as applied to DNA analysis, can not only be utilized to provide identifications of individuals, they can also be used to link suspects to scenes, victims to suspects, and crimes to one another. There is no question that DNA has revolutionized the analysis and comparison of physical evidence. It is a powerful tool in the forensic repertoire that has certainly impacted innumerable cases and the lives of countless individuals. It is, however, only one of the many scientific tools that are available to assist with criminal investigations.

The meteoric rise of DNA analysis, along with its vast string of successes, has fostered a new attitude whereby investigators and attorneys alike are taking the stance that if you do not have DNA evidence, you do not have a case. The corollary of this sentiment, which is equally as dangerous, appears to be taking hold as well; whereby it is not uncommon for investigators to concentrate on and collect only biological evidence while large amounts of other types of physical evidence goes overlooked. These attitudes, bolstered by a recent spate of articles in the peripheral literature, have placed an over reliance on DNA that has cast dubious shadows on traditional areas of forensic analysis including but not limited to fingerprints, firearms, toolmarks, and trace evidence. A fissure has opened splitting DNA evidence from what has now been commonly referred to as "non-DNA evidence".

The above listed "non-DNA" fields have historical roots and have been the proverbial bread and butter of the forensic sciences for a relatively long time. These fields produce somewhat subjective results that require a great deal of experience to interpret. Due to this reliance on experience, in combination with established methods for quantifying DNA results, there is an unfair perception that the results obtained from fingerprints, firearms, toolmarks, and trace evidence are unreliable. Based on such unfortunate comparisons there are now various movements, both patent and latent, that are attempting to discredit these fields in their entirety. This is most unfortunate due to the fact that, as a result of such efforts, there is now an abundance of good evidence that is not being utilized.

The goal of this presentation is to address the significance of this new paradigm by setting forth and answering several questions: 1) What is the role of the scientific method in the forensic sciences? 2) What is the general basis for the fissure between DNA and non-DNA evidence? 3) What is more important in an investigation DNA evidence or non-DNA evidence? and 4) Does non-DNA evidence still have an important role in the forensic sciences?

In answering these questions, a philosophical discussion on the role of non-DNA evidence in criminal investigations, with an emphasis on the value of trace evidence, will be provided. The point of view to be presented will emphasize a collaborative approach to the scientific investigation of criminal activities whereby all relevant evidence should be evaluated and included in the theory forming process. After all, a theory is only as good as its supporting evidence. The more evidence that is taken into consideration, the better the refinement process and the stronger the theory will become. By taking such a collaborative approach to the scientific investigation of crimes and incorporating as much factual information as possible from as many disciplines as possible, a proposed theory will be much stronger and will stand up to ever greater challenges.
**Non-DNA Evidence, Trace Evidence, Scientific Investigations**

## A69 Evolution of "The Method" — The Past, Present, and Future of Forensic Intelligence

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After attending this presentation, attendees will understand that as forensic science becomes more sophisticated, it also seems to become harder for investigators to access. This presentation will illustrate the importance of investigative forensic science otherwise known as forensic intelligence.

This presentation will impact the forensic community by explaining how forensic science is primarily being used as an adjudicative tool in the criminal justice system. This presentation will allow attendees to see the value of investigative forensic science and better understand its history.

Upon their first meeting, the slender man with the intense stare and the eagle like face pronounced "you came from Liberton. You drive two horses, one gray, and one bay, and you are probably employed by a brewery." After all the assumptions were confirmed and the man from Liberton left, the slender man explained himself. "I saw the clay from Liberton on the fellow's boots. He had gray hairs on one sleeve and bay hairs on the other. As for my final bit of deduction, you probably observed the face, especially the nose."

While this exchange has all the hallmarks of the most famous fictional detective, it was neither Sherlock Holmes nor fictional. It was only one of many accounts of the keen observational and interpretative skill of a Scottish physician named Dr. Joseph Bell. This account was originally recorded by Hesketh Pearson and then obtained by Ely Liebow for his work on Dr. Bell's biography. Dr. Bell used what he termed as "the method" every day as he treated patients and taught medical students like the young Arthur Conan Doyle.

Dr. Bell's keen insights did more than just help his patients and provide the inspiration for Conan Doyle to create Sherlock Holmes. Since the 1870's, Dr. Bell used his talents to aid the crown with criminal investigations. He continued on this path for nearly 20 years. For a time he was even involved with the Jack the Ripper investigation. The discretion Dr. Bell exercised regarding his involvement in these cases has resulted in a lack of appreciation for the central role he played in fostering the scientific investigation of crime.

As more of the historic literature in Forensic Science is explored, it becomes apparent that forensic science was not only used at trial, but as an active part of the investigation to develop suspects. Similar feats of observational prowess and interpretive intellect are present in works from Hans Gross and Edmund Locard, to Paul Kirk. Yet somehow over time the laboratory became removed from the investigation assuming a more reactive role. The scientist was detached from the inception of the investigation at the crime scene. Slowly, the concept of a general knowledge of forensic science or a "generalist" started to give way to the concept of a laboratory specialist.

Somewhat ironically, the juggernaut specialization of DNA has once again illustrated the investigative power that comes from the proper interpretation of physical evidence. Now more than ever, physical evidence is being used to develop suspects where the traditional investigation had failed. While on the surface, the genetic information contained within biological evidence seems to offer the most potential, there are times when it is useless without other basic information. A basic knowledge of bloodstain patterns allows investigators to select bloodstains at a crime scene that might be foreign to the victim. Other aspects of physical evidence are critical to fully understand the significance of the biological evidence that has been recovered.

In the traditional criminal justice system, change can be cripplingly slow; however, there are times when outside events can provide unforeseen opportunities for the advancement of the science. As the United States is actively involved in the war against terrorism on several fronts, the need for real-time information about our adversaries has led us back to physical evidence. Fingerprints, DNA and other physical evidence found at the scenes of roadside bombs now can tell us just how many people are involved in setting the devices and help troops identify those responsible. Battlefield Forensics is allowing the traditional criminal justice system to see just how useful forensic science can be as an investigative tool.
**Forensic Intelligence, "The Method", Investigative Forensic Science**

## A70 The Tyranny of the Machine and the Role of the Criminalist

*Peter D. Barnett, BS\*, Forensic Science Associates, 3053 Research Drive, Richmond, CA 94806*

After attending this presentation, attendees will understand the role of the criminalist in the identification of physical evidence at the scene of

an incident and the development of relevant conclusions concerning that evidence once the analysis of the evidence has been completed.

This presentation will impact the forensic community in an effort to convince criminalists that their role is not primarily the operation of automated analytical machinery, but in the recognition of physical evidence and the testing of relevant hypotheses based on the analysis of that evidence.

The modern forensic laboratory is a place where scores of people monitor dozens of machines that use terabytes of flops to generate gigabytes of data resulting in megabytes of results printed on reams of paper finally resulting in a three page report with conclusions which are often ambiguous, irrelevant, incomplete, or misleading. What is missing in this process? This presentation will look at the input to the laboratory, and the output from the laboratory and demonstrate that often the input is deficient and the output does not address the relevant questions. From the crime scene to the analytical machine, and from the analytical data to the opinion of the forensic scientist, the laboratory machinery is irrelevant. At the incident scene, scientists need consider basic principles of criminalistics (Transfer, Individuality, and Divisibility) to understand how physical evidence is produced. Scientists must understand basic principles of chemistry and physics to understand how evidence may be altered as it is produced, by the passage of time, or as a consequence of the scene environment. After the data is produced by the laboratory machinery, the scientist must understand the basic processes of scientific inquiry (Deduction, Induction, and Abduction) to derive a hypothesis and design an experiment to test that hypothesis. And finally the *forensic* scientist must understand applicable legal requirements (admissibility, expert testimony, and applicable statutory and case law) to insure that the relevant questions have been addressed. The critical component in both the pre-analysis scene investigation and the post-analysis reporting is the scientist. Case examples include an automobile accident in which one of the two occupants survived, and was charged with manslaughter. Blood samples taken from the very badly damaged car, but the report did not address with any specificity how the samples that were subjected to DNA profiling could be used to establish who was driving the car. In another case, the female occupant of a vehicle was fatally shot, either by the driver of the vehicle or a person standing outside of the vehicle. GSR was found in multiple locations of the vehicle and the clothing of the driver. Reenactments of the possible positions of the shooter were done, but the analyst provided little information to the jury to allow them to determine which alternative was more likely. In another homicide case, fibers were essentially the only evidence. A wide variety of analytical methods were used to measure various properties of the evidence and exemplar fibers. No effort was made to determine the relative or cumulative value of the techniques that were employed. Further, no effort was made to determine a reasonable explanation for a connection between the evidence fibers and the alleged crime. In each of these cases, in spite of extensive and very sophisticated analytical work, little guidance was provided to the investigators or jury as to how the evidence related to the questions that were relevant to the adjudication of the case. Isn't that our job?

**Criminalistics, Evidence Recognition, Hypothesis Testing**

## A71 The Use of Direct Analysis Mass Spectrometery for Ink Analysis

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The goal of this presentation is to determine if the direct analysis mass spectrometery is sufficient to analyze various types of inks.

This presentation will impact the forensic community by teaching about new applications of the direct analysis mass spectrometery of inks.

The most widely encountered material found on documents submitted for forensic examination is ink. All inks, in their basic form, are mainly composed of a colorant(s) that is suspended in a vehicle (solvents and resins). There are also other ingredients that may be present in inks, which can include antioxidants, preservatives, and trace elements, but these typically form a small fraction of the overall formulation. Nevertheless, their importance should not be discounted because it is possible that the combination of these components allow otherwise similar inks to be discriminated. There are different types of inks that may be encountered on documents such as writing inks, printing inks from conventional commercial printers, and office machine inks. The results from an ink examination can be extremely beneficial by helping link multiple documents, ascertain whether they have been altered, and determine when they were produced.

The mass spectrometer used is capable of performing direct analysis in real time and allows spectra to be obtained by placing a sample in the path of the ion source. The methodology can be virtually non-destructive and involves very little sample preparation. Although introduced in 2005, numerous applications have been documented in the recent literature with promising results for the analyses of various materials. In particular, research has been published in the area of ballpoint writing ink analysis. This study will evaluate sample preparation and focus on optimizing parameters for various types of inks including ballpoint, non-ballpoint, and inkjet inks. Individual components and the entire formulation of the inks will be analyzed using the non-destructive method as well as liquid extractions. Spectra obtained from neat samples will be searched against a library composed of spectra from the individual components to determine the feasibility of identifying characteristic ions that make certain inks unique. Finally, inkjet ink documents, which can be composed of multiple colors (e.g., cyan, magenta, yellow, black, light cyan, light magenta) will be analyzed to determine if the mixture of inks can be compared with combined individual spectra in an attempt to identify a particular manufacturer.

The results from this study will lead to a greater understanding of the analysis of inkjet and writing inks. The analytical data obtained from this technology may potentially have a significant impact on the conclusions reached in cases that involve the comparison and identification of inks.

**Direct Analysis in Real Time, Inks, Mass Spectrometry**

## A72 Analysis of Pigmented Inks by Pyrolysis Gas Chromatography - Mass Spectrometry

*Jay A. Siegel, PhD, Lilyvet Rivas, MS, and John V. Goodpaster, PhD\*, Indiana University Purdue University Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the nature of pigmented inks, their role in criminal activity, and how pyrolysis gas chromatography can be used to differentiate pigmented inks by manufacturer and type.

This presentation will impact the forensic community by showing how pigmented inks on questioned documents can be analyzed and by providing a new analytical tool in questioned document analysis.

Personal computers and associated printers are used in a variety of crimes today. These include counterfeiting (money and identification documents such as drivers licenses, passports, and birth certificates), child pornography, threatening letters (including chemical and biological threatening hoax letters), financial contracts, wills, and criminal record keeping (drug dealers and terrorists). Many of these have increased greatly owing to heightened terrorism activities. Because of all of this illegal activity it becomes increasingly important for law enforcement and antiterrorism agents to have the most modern tools for combating these threats. These tools include state of the art capabilities in forensic science laboratories for characterizing and associating items of physical and scientific evidence. As the examples above show, the analysis of inks used in writing instruments and computer printers is of paramount importance.

Pigmented inks are among the most popular inks used in ink jet printers today. They have also been extensively used in some types of writing instruments including certain gel pens. Ink jet printers are inexpensive and produce high quality photographs as well as documents. They are the most popular printers used today on personal computers in homes and offices. As a result, documents and photos produced by these printers are becoming common types of evidence in cases involving document fraud including photos, art works and other documents. Pigmented inks consist of tiny particles of colorant suspended in a vehicle (solvent plus additives), which are then sprayed onto a paper surface. Almost no research has been done to characterize pigmented inks in a systematic way. In this research a battery of instrumental techniques will be used to characterize these inks. The techniques will include pyrolysis gas chromatography/mass spectrometry, matrix assisted laser desorption mass spectrometry, thermogravimetry and differential scanning calorimetry. A collection of 150-200 specimens provided by the US Secret Service Laboratory (who suggested part of the proposed research) will be used in this research. These techniques will also be used to explore how solvent content in the ink on a paper plug can be used to determine the age of the ink. A website will be developed that will make the searchable library and all of the mass spectral and thermal data available to the forensic science Pigmented inks solubility in water and organic solvents makes them difficult to analyze using routine methods. These inks are resistant to water and mechanical abrasion and tend to fade more slowly than dye-based inks. For all these reasons pigmented inks are in great demand and are being used frequently in inkjet printers and gel pens. This popularity has resulted in writing instruments using pigmented inks to show up as evidence in questioned documents cases. Pyrolysis-gas chromatography-mass spectrometry (py-GC/MS) is an analytical technique that allows the analysis of insoluble, non-volatile solid materials that are difficult to analyze by routine methods such as thin layer chromatography (TLC). The problem addressed here is whether or not py-GC/MS could be used to distinguish among various specimens of pigmented inks on paper. Ninety-three pigmented inks have been analyzed by py-GC/MS. The resulting chromatograms were analyzed applying multivariate chemometrics. After pretreatment, hierarchical clustering analysis, principal components, and discriminant analysis were applied. These techniques were used successively so that an objective and reproducible discriminant model was calculated in the final step.

**Pigmented Inks, Questioned Documents, Pyrolysis Gas Cheomatography**

## A73 Evaluation of Capabilities of Laser Ablation ICP-MS for the Forensic Analysis of Gel Pen Inks

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The goal of this presentation is to provide the forensic community with a critical evaluation of the value of using LA-ICP-MS for the elemental profiling of gel inks.

This presntation will impact the forensic community by proposing an innovative method for the forensic examination of gel inks. The use of LA-ICP-MS on inks will also expand the number of matrices currently analyzed by this technique in trace evidence and will facilitate the use of this methods in forensic laboratories.

Gel pen inks have become a prominent type of ink found in forensic document examinations due to its favorable chemical and physical characteristics and low cost of manufacture. Nevertheless, the analysis of gel pen inks constitute a challenge for the forensic ink examiner since most of the gel inks are difficult to analyze by conventional techniques such as paper chromatography, TLC and capillary electrophoresis.

As a result, other non-destructive or less-destructive methods such as Ramman spectrometry, infrared spectroscopy and XRF have been recently explored as alternative tools to cope with forensic comparisons of gel inks.

The purpose of the present work is to conduct method development and evaluation of the capabilities of LA-ICP-MS for the qualitative and quantitative elemental analysis and comparison of gel inks.

Laser ablation is a leading technology for direct solid sampling and has become a valuable tool for elemental analysis in forensic science. The technique has been successfully used for forensic analysis of glass, paints, soils, diamonds, gold and other matrices. Some of the advantages of LA-ICP-MS include direct characterization of solids, elimination for the need for chemical procedures for dissolution, minimum consumption of the sample (~nanograms), high sensitivity and high selectivity.

These advantages make LA-ICP-MS a very attractive technique for forensic analysis, especially for ink examinations where the amount of sample always represents a challenge and quasi non-destructive methods are important.

A comprehensive evaluation of the capabilities and limitations of this novel technique are presented in this work. Evaluation of parameters

of forensic interest is discussed in detail, including the analytical performance of the technique, accuracy, precision, discrimination potential, homogeneity of the ink and the paper at micro-scale, reproducibility, sampling size requirements, data analysis and interpretation of results.

Laser ablation was optimized in low density energy mode in order to minimize the destruction of ink and its supporting media. Qualitative determinations were conducted on a set of 45 black gel inks in order to evaluate its discrimination power and to identify the most informative elements. Quantitative analysis of the samples was also conducted by LA-ICP-MS to better characterize the elemental profile of the unknowns.

In-house matrix match standards were designed to conduct quantitative determinations. Different papers (Whatman 2, 42 and 542) were tested as the support matrix for the preparation of the ink standards. Fountain pen black ink was spiked with a large suite of elements and analyzed by acid digestion ICP-MS and LA-ICP-MS. Excellent correlation was obtained between the concentration of the ink obtained by acid digestions and LA-ICP-MS.

External calibration and standard addition methods were used to characterize the ink standards. Good accuracy and precision were obtained at different spike levels (%bias <13%, %RSD <5%). Good linearity was achieved for the standard calibration curves where the amount of ablated ink ranges between 0.8-8 pg.

Scanning Electron Microscope images and elemental analysis by SEM/EDS and XRF were used to assist the method development and characterization of the ink standards.

A study on different papers commonly found in forensic document examinations was also conducted to further evaluate the applicability of the method to real case scenarios. The proposed method could be potentially extended to other type of writing inks to enhance discrimination and classification of inks.

**Inks, Trace Elemental Analysis, Laser Ablation**

## A74    Microanalytical Characterization of Architectural Paint Tinting Pigments

*Christopher S. Palenik, PhD\*, Heidi Bonta, BS, and Skip Palenik, BS, Microtrace, 790 Fletcher Drive, Suite 106, Elgin, IL 60123*

After attending this presentation, attendees will have a better understanding of the chemistry of architectural paint pigments.

This presentation will impact the forensic community by providing background information about the composition of architectural tinting pigments.

The coloration of modern architectural paints is achieved through the addition of specific ratios of tinting pigments. This presentation discusses the major tinting pigments used in modern architectural paints in the context of forensic paint evidence.

Tinting pigments are introduced as viscous colored liquids through manual or automated aliquots of specific volume to a white base paint to achieve specific custom colors. Although coloration pigments are typically present at low levels, they can be recognized through examination by polarized light microscopy (PLM) and identified through a combination of microanalytical methods. The most applicable technique for any given paint is dependant upon the actual pigments in a given sample. Therefore, a thoughtful (*i.e.*, scientific) approach to pigment identification that is modified to fit the sample at hand is critical to the evaluation of pigment evidence.

To cover a wide range of color space, manufacturers have developed a set of 12 "universal" tinting pigments. This set of 12 pigments is compatible with oil and water based paints produced by most major paint manufacturers. The universal tinting pigments include the nominally described colors: black, white, green, blue, red, magenta, yellow (3 shades), and brown (3). At least one manufacturer uses its own set of 12 tinting pigments. This manufacturer-specific set substitutes gray and orange shades in place of a brown and yellow.

Three sets of universal and one set manufacturer-specific tinting pigments have been acquired from various home improvement stores. The pigments and fillers present in these densely colored solutions have been isolated through a series of sonicated acetone washes. The dried solids were prepared for analysis by a variety of microanalytical techniques for the purpose of characterizing the solid contents of these tinting solutions.

The techniques used to study the isolated solids in this work include PLM, Raman microspectroscopy (RMP), energy dispersive X-ray spectroscopy (EDS), fluorescence microscopy, visible microspectrophotometry, and X-ray diffraction (XRD). Although XRD is not a microanalytical technique, it is helpful in establishing the initial identification of fillers used in the tinting pigments. Similarly, without extensive sample preparation, EDS generally does not provide a way to look directly at individual pigments in paints; however, the bulk elemental composition can provide evidence as to the major pigments in use.

The architectural tinting pigments studied contain a range of inorganic and organic pigments. The black tinting colors use carbon black, while the whites use rutile ($TiO_2$). In general, one yellow and all brown pigments are based around the iron oxides goethite (FeOOH) and hematite ($Fe_2O_3$). Copper phthalocyanines are used as the blues (PB 15 – chlorinated) and green (PG 7 – brominated/chlorinated). The red, orange, magenta and yellow (2) are based around other organic pigments. In addition, all of the tinting pigments contain significant amounts of inorganic fillers such as talc, calcite and other silicates. Using the aforementioned analytical techniques, some tinting pigments can be distinguished by manufacturer.

This presentation discusses the results of our research into the background of these architectural tinting pigment sets, the results of our analysis and applications of this information to forensic paint analysis.

**Paint, Pigments, Microscopy**

## A75    Analysis of Automotive Paint Clear Coats by UV-Visible Microspectrophotometry, Raman Spectroscopy, and Fourier Transform Infrared Microspectrophotometry

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After attending this presentation, attendees will understand how automotive clear coats are analyzed for forensic purposes and the ability

of UV microspectrophotometry, laser raman spectroscopy, and infrared microspectrophotometry to discriminate among clear coats.

This presentation will impact the forensic community by providing a new analytical tool in the characterization of automotive clear coats.

The purpose of this research project is to evaluate several analytical techniques for their ability to discriminate among automotive clear coat finishes. Automotive paints have been important examples of trace evidence in crime laboratories for many years. Paint chips and/or smears are found at many automobile crash scenes including those involving multiple automobiles or a vehicle and a pedestrian. Paint evidence may be transferred from one car to another or to clothing or the body of a pedestrian. Automobile paint consists of several layers. These include one or more primer layers which serve to provide a good adhering surface for subsequent layers and often to provide rust protection. Over top of primers are topcoats (color coats) which give the finish its color and help protect the body of the car. Since the early 1990s, car manufacturers have been adding clear coats to their paint finishes. The clear coats consist of a film former and one or more light scavengers. The clear coat protects the topcoats from scratches, dents, breaks and the ravages of ultraviolet light. Forensic analysis of automotive paints involves a series of visual and analytical tests that may be done on the paint as a whole, with all of the layers intact or on individual layers. Such tests include analysis of the layer structure including thicknesses and color of each layer. Other tests are used to determine the chemical structure of the binders and pigments present in individual layers of the paint. Tests include pyrolysis-gas chromatography, infrared spectrophotometry, visible spectrophotometry and, more recently, raman spectroscopy. Most of the analysis of paints focuses on the topcoats and on the primers. Until recently, very little work has been done on clearcoats. In this project, a number of analytical techniques were evaluated for their ability to discriminate among clear coats. More than 200 samples of automotive finishes were obtained with make, model and year data, from paint and body shops and junkyards. These were sectioned and the clear coats isolated. They were subjected to UV microspectrophotometry, laser Raman Spectroscopy and infrared microspectrophotometry. The data collected was analyzed using statistical techniques including clustering, principle component and discriminate analysis. This paper reports mainly on the results obtained with UV microspectrophotometry. Results show that this technique is useful in discriminating clear coats.

**Automobile Paint, Clear Coat, UV Microspectrophotometry**

## A76 Statistical Pattern Comparisons of Striated Tool Marks: Defending Against Daubert and Frye Challenges

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After attending this presentation, attendees will understand the application of computers and statistical pattern recognition techniques to areas of interest to practitioners of tool mark and firearms analysis.

This presentation will impact the forensic community by showing how practitioners can apply well documented statistical pattern recognition methods to striated tool mark evidence in order to make numerically based identifications and compute estimated error rates. Such abilities are necessary to defend the well known, but (largely) statistically untested conclusions in firearms and tool mark analysis. *Daubert* and *Frye* challenges to the admissibility of scientific trace evidence analysis are major issues for forensic science.

Given a set of tool marks with the same class characteristics, how likely is it, that when comparisons are made between the subclass and individual characteristics, a tool mark is misidentified? With recent challenges to the admissibility of tool mark evidence such a question is of paramount importance. In this presentation, it will be shown how methods of statistical pattern recognition (i.e., machine learning) can significantly aid in answering this question. Also, because these methods have been published and they allow for the computation of error rates, they can satisfy both the *Frye* and *Daubert* standards for admissibility of scientific evidence.

In this study, modeling clay is used to generate reproducible sets of striation patterns made with several standard tip screw drivers for slotted screws. The striation patterns are photographed under the stereo microscope and various measurements are made with the digital image processing software. Using these measurements, the striation patterns can be encoded into a feature vector. In combination with various Hilbert space kernels, we process the resulting feature vectors with principal component analysis (PCA), Fisher linear discriminant analysis (FLDA), maximum likelihood Gaussian classifiers (MLGC), support vector machines (SVM) and neural networks (NN). The advantages and drawbacks to each of the above statistical methods in the context of forensic tool mark analysis will be presented. Error rates, classification confidence intervals and credibility measures are estimated from the hold-out method and conformal prediction theory. Finally, how all the statistical methods presented in this talk can easily be extended to any set of tool marks made by virtually any tool will be discussed.

**Tool Marks, Statistics, *Daubert* and *Frye***

## A77 SEM Analysis of Saw Marks in Bone

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After attending this presentation, attendees will learn a useful technique to assist in the evaluation of saw marks found on large areas of bone or in cases where multiple saw blades from the same class need to be examined.

This presentation will impact the forensic community by assessing the capabilities of the forensic identification of saw mark patterns in bone as well as the impression technique using PVS and epoxy resin for SEM analysis. This information could be used to assist in the evaluation of saw marks found on large areas of bone or in cases where multiple saw blades from the same class need to be examined.

In situations in which a victim's body is mutilated in order to prevent identification after a murder, investigators are sometimes faced with the task of identifying dismembered remains. With advances in DNA analysis and forensic odontology, positive identification of dismembered remains is increasingly common, but it is of further interest for the

investigator to determine what type of tool was used for such an act, in hopes of comparing tool mark patterns left behind in the bone with evidence found at a crime scene or in a suspects possession.

Although there are many types of saws or tools that are available for this type of act, one tool that is high powered, easy to use and accessible is the reciprocating saw, which is the focus of this study.

It has already been determined that reciprocating actions of saws tend to enhance rather than erase characteristics necessary for saw class identification, but it is of importance to be able to further categorize a saw used to commit a crime by type characteristics. This information is usually recorded in the object being cut, such as bone, and can help to determine if a particular questioned saw blade made a saw mark pattern so alike to a known saw blade pattern, that they can be considered a match.

It was one goal of this study to be able to determine whether or not 10 identical reciprocating saw blades from the same class would leave behind saw mark patterns that exhibit individual characteristics. In other words, if all 10 saw blades were worn in the exact same manner and then used to make a cut into bone, is it possible that each blade would possess uniqueness enough to identify which blade made a particular mark in the bone?

In order to determine if this was possible, cadaver skulls were cut into 1.5" by 1.5" sections, which were then labeled to coincide with each saw blade. A typical 14.2 volt cordless reciprocating saw with a 7/8" stroke and 2,700 strokes per minute was used along with ten 14 TPI, 6" bi-metal cutting blades in order to create saw marks in the bone.

Furthermore, there were several saw blades that were worn with a varying number of cuts, from several hand strokes to simulate hesitation cut marks, to many reciprocating saw cuts to mimic that of a severely worn blade. As the teeth on the saw blade were worn, the striation patterns left behind on the object being cut varied.

One common method of analysis for tool marks in bone is microscopy. Yet, whether or not a tool mark is said to match a particular tool is often subjective and left up to the discretion of the examiner due to the fact that there are many characteristics to be determined from a saw mark pattern including blade and tooth size (TPI), set, shape, power and cutting directions. Since there are currently no set standards or protocol as to how many aspects must coincide in order to determine a match between a questioned saw mark pattern and a known saw blade pattern, there has been an increasing lack of trust in the forensic field of tool mark examination and analysis.

Scanning Electron Microscopy (SEM) is a useful method for analysis of saw marks in bone due to its ability to produce images of high-powered magnification. This instrument allows one to take a closer look at the kerf, or sawed groove left behind in the bone and also makes it possible to identify striations and micro striae, which are patterns left behind on the kerf walls of the cut that record the blades stroke. With this information it is likely to determine if the questioned saw mark in the bone matches the saw mark from a known source. It is the unique characteristics that each saw blade retains that can drastically reduce the chances of any other blade making a particular cut.

However, there are disadvantages involved with using SEM for the traditional analysis of tool marks in bone. The instrument itself can only hold samples up to 3" in size, which poses a problem in situations where long bones or large saw mark patterns need to be analyzed. Additionally, the moisture in bone can cause problems with the sensitive closed vacuum chamber needed for the proper operation of SEM. In order to eliminate these potential problems, a negative-positive impression technique was used.

First, negative impressions were taken of each bone sample containing the saw marks using polyvinylsiloxane (PVS), followed by positive impressions using epoxy resin. This method allowed for a replica to be made of the bone and the saw mark pattern present in each sample and yet offered a practical use with SEM. SEM images were taken for each saw mark impression and for each saw blade sample. Digital and optical pictures of the samples were also recorded and used for comparison.

It was another goal of this study to assess the impression technique using these materials in order to determine if the saw mark pattern was completely preserved throughout the process. By comparing images of the saw mark patterns in the actual bone samples with the impressions made, it was possible to establish whether or not the type characteristics had changed throughout the duplicating process.

Forensic identification of saw marks in bone involves detailed steps of comparing a questioned piece of evidence to a known saw type in order to form an opinion as to whether the items are similar enough to be called a match. A forensic tool mark examiner must then evaluate to what extent those items are said to match by determining the probability that the questioned saw mark and known saw mark were derived from the same saw blade.

Both similarities and differences in the saw mark patterns from the various saw blades used in this experiment were expected. This study will impact the forensic community by assessing the capabilities of the forensic identification of saw mark patterns in bone as well as the impression technique using PVS and epoxy resin. This information could be used to assist in the evaluation of saw marks found on large areas of bone or in cases where multiple saw blades from the same class need to be examined.

**SEM, Saw Mark Patterns, Impressions**

## A78 Examination of Electronic Control Device Probes to Determine Duration of Application

*Richard T. Wyant, MS\*, Washington State Patrol CLD, 2203 Airport Way South, Suite 250, Seattle, WA 98134; and Andrew Hinz, BS\*, TASER International, 17800 North 85th Street, Scottsdale, AZ 85255*

After attending this presentation, attendees will learn the usefulness in collecting Electronic Control Device related evidence and how Scanning Electron Microscopy can be used to determine if and how much electrical energy is delivered to a subject during a electronic control device application.

This presentation will impact the forensic community by presenting data that will be useful in the investigation of accusations of excessive force and in-custody deaths involving the use of electronic control devices.

Electronic Control Devices (ECD)have become standard equipment for many law enforcement agencies across the US. Their primary purpose is to deliver an incapacitating shock to a subject through two wire-tethered probes connected to the unit. These devices are used daily with a high degree of success, but when they are not successful, ECDs receive substantial scrutiny. Often when an excessive force complaint is filed, or if an in-custody death occurs, forensic investigators are

summoned to reconstruct the event. Historically, the main tool for the investigator has been the data available from the unit itself. Some ECD units have on board memory that records the activation time and duration of the unit. This data, however, does not necessarily equate to the duration of electric energy delivered to the subject.

When an ECD is activated, current across a small primer in the ECD cartridge ignites forcing a nitrogen capsule rearward into a hollow puncture pin. The compressed nitrogen is released into two chambers forcing the blast doors, probes, probe wires, and Anti-Felon Identification (AFID) tags forward out of the cartridge. The two aluminum probes/darts, attached to thin insulated wires, impact into a target. If the probes are typically within 2 inches of a conductive target, electrical energy will be transferred between the two probes completing the electrical circuit. The completed circuit delivers pulses (energy) through the target in an attempt to temporarily incapacitate a human target.

For energy to be transferred from the device, both probes must simultaneously contact the subject to complete the electrical circuit. The probes can miss the target or become dislodged during the incident. If this occurs, the electrical energy will complete its circuit across the wires or by arcing in front of the electrodes of the ECD. Energy will not be delivered into the target.

The wire is connected to the probe by a single knot tied at the base of the probe. At the wire/probe junction, the electric spark jumps between the wire knot and the probe (air-gap) completing the circuit. Due to the impedance of the air-gap, this jump creates heat resulting in melting, scoring, and carbon residue deposits on the knot and the inner surface of the probe from the thermal insult.

Twenty-five ECD cartridges were fired into conductive media at 1-, 5-, 10-, and 20-second durations. The probes from these cartridges were examined stereo microscopically and with the scanning electron microscope (SEM). The physical changes (carbon residue, melting, scoring, and pitting) on the wire knots and the inner probe surface were measured and quantified. It was determined with a high degree of confidence that analysis of the physical changes and residue left on the probes and wire knots can glean information on the duration of electrical energy actually delivered to the subject.

By using stereo and electron microscopy to document, measure, and quantify the physical changes of the ECD probe/wire junction, accurate data on the amount of electrical energy delivered to the subject can be determined within the 1-, 5-, 10-, and 20-second timeframes. If the evidence is properly collected and documented, qualities present on the probes can be compared to the data recorded in the ECD unit itself to determine the duration of electric energy the subject received during the incident.

**Electronic Control Device, In-Custody Death, Electricity**

## A79    Individualization of Evidence

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After attending this presentation, attendees will understand the nature of the concept of individualization of evidence and the arguments for and against labeling any evidence as being individualized

This presentation will impact the forensic community by providing further understanding of the concept of individualization and the conditions under which it may be proper to use it

In this session, a panel of experts will examine the notion of individuality of evidence; the idea that a piece of physical evidence can be associated with one and only one object or person. What proof is there for the contention that pattern evidence such as fingerprints, bullets, shoe prints, and handwriting can be linked unequivocally to one source to the exclusion of any other? Is it a proper application of statistics to infer individuality of DNA evidence on the basis of the product rule?

The process of classification of physical evidence consists of putting it into successively narrower classes of objects. The goal is ultimately to put the object into a class of one. This is called "individualization". It is the "….science of individualization" (James W. Osterburg). For nearly a century testimony has been offered and accepted in court that a fingerprint obtained from a crime scene and developed by powder or chemical techniques can be unequivocally associated with a single finger on a single person. Similar expert testimony has been offered on behalf of bullets being linked to a specific firearm, handwriting to a particular person, shoe prints to a single shoe, etc. Conclusions of individuality are reached through empirical observations. Practitioners have examined large numbers of exemplars and conclude that, for example, bullets fired from one gun have unique markings which may be associated with one gun to the exclusion of all others. With fingerprints, for example (and this argument is made for other types of pattern evidence), experts may claim that fingerprints are a result of random processes, and proffer the notion that nature doesn't repeat herself (often stated as: all snowflakes are unique).

But are these notions really defensible – scientifically? mathematically? logically? Or does belief in individualization rest more on faith and cognitive illusions? Can any sample of observations prove uniqueness for an entire population of objects? Can probability theory ever support claims of unique individuality? The founders of various individualization fields put forward rationales that supported probabilistic conclusions while encouraging absolute conclusions. Increasing numbers of forensic and other scientists, as well as statisticians, argue that the data are insufficient to support the traditional theory and the theory itself is incoherent. More recently, the use of statistics in forensic DNA testing has become the *de facto* means to "individualize" biological evidence back to a subject. The use of statistics does not prove the evidence is

unique but rather, it is argued, demonstrates that the chances that the DNA came from some other person are so small that it is unreasonable to consider – an argument which concedes the essential problem at the same time that it seeks to sidestep it).

We will examine these ideas and hopefully shed light on the contention that individualization of evidence in forensic science is a fallacy. If individualization is indeed an unachievable aim, and probabilistic inferences are the only reality, how can the forensic science constructively adjust, not only in its theory but in its practices?

**Individual Evidence, Scientific Evidence, Admissibility of Evidence**

## A80 The Development of a Screening Method for Biological Samples Using a Real-Time PCR Assay for HLA-DQA1

*Lawrence Quarino, PhD, and Allison G. Taylor, BS\*, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

The goal of this presentation is to describe the development of a screening method for biological samples using a real - time PCR assay for HLA-DQA1.

This presentation will impact the forensic community by providing an analytical method to those in the forensic biology community that will screen DNA samples for possible common origin.

In laboratories with limited resources, a DNA screening test to identify potential samples from a common origin would be of great benefit. Differentiation of bloodstains from different sources on a sample of biological evidence is often based on bloodstain pattern analysis which could be problematic to forensic biologists who may not have the experience or training to make the correct interpretation. The development of a DNA screening test using real-time PCR was attempted by examining differences in the slopes of melting curves and first derivative melting curves, and the melting temperature of HLA-DQA1 amplicons from samples from individuals with varying HLA-DQA1 genotypes. HLA-DQA1 was chosen as a screening locus to develop the screening method because its polymorphism is based on sequence creating the likelihood of melting curve and melting temperature variation as a function of genotype. In addition, polymorphisms from HLA-DQA1 are well established considering that the HLA-DQA1 locus was once routinely used in forensic DNA typing. The HLA-DQA1 locus was amplified in buccal swabs from several individuals with known HLA-DQA1 genotypes using a real-time PCR assay utilizing SYBR green to measure PCR product through fluorescence. Samples were amplified on a Corbett RotorGene 6000 using the following PCR parameters: $95^0$C hold for 10 minutes to activate the polymerase, $95^0$C for 15 seconds, $55^0$C for 30 seconds, and $72^0$C for 30 minutes for 40 cycles. Subsequent examination of melt curves and the melting temperature of amplified product show that variation does occur with HLA-DQA1 genotype and replicate results from the same genotype are reproducible. Results thus indicate that HLA-DQA1 can serve as a screening locus to help locate biological samples from different sources prior to the development of a DNA profile. Additional study is also being conducted to determine the possibility that this assay could simultaneously quantitate high copy DNA samples such as bloodstains.

**DNA Screening Method, Real - Time PCR, HLA-DQA1**

## A81 Development of a Forensic Screening Tool Using STR DNA Analysis

*Katie Oostdik, MS, Dawn R. Rabbach, Patricia M. Fulmer, PhD, Cynthia J. Sprecher, BS, Rita Weispfenning, PhD, Melissa R. Schwandt, PhD\*, Julia Langbehn, and Douglas R. Storts, PhD, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711*

After attending this presentation, attendees will understand the utility and performance of STR screening kits in forensic DNA typing and sample screening applications.

This presentation will impact the forensic community by introducing the concept of STR DNA analysis used as a screening tool to increase efficiency in Forensic DNA Laboratories.

Multiplexed Short Tandem Repeat (STR) analysis has become the dominant technology in DNA-based human identification. As the number of samples typed per case increases, especially in complex homicide or sexual assault cases, the need for less expensive methods for screening these multiple samples becomes apparent. By using a simple yet extremely sensitive STR system, the forensic DNA laboratory can quickly discriminate between the limited number of donors present in a given case. In addition, large populations of potential donors can be mass-screened inexpensively at a level of discrimination sufficient to identify only a very small number of possible matches. Following this, the laboratory can then select the most probative DNA samples to continue with a full compliment of STR testing.

An STR screening kit has been developed for the co-amplification and two-color-detection of 4 STR loci: (D18S51, D8S1179, TH01 and FGA) and amelogenin. The amplicon lengths of the largest loci have been significantly shortened so that all amplicons are less than 260bp. The robust and careful design of this screening kit provides maximum sensitivity with low quantities of DNA (less than or equal to 50pg). This makes the system ideal for use with low copy numbers samples including touch samples. The reduced number of loci in the screening kit provides sufficient data for screening purposes at an economical price point. Based on U.S. Caucasian population frequency estimates, this STR screening kit has a power of discrimination (PD) of approximately $1.9 \times 10^5$.

In this multiplex, one of the two primers for amelogenin, D18S51 and D8S1179 are labelled with fluorescein and one of the two primers for TH01 and FGA are labelled with 6'-carboxy-4',5'-dichloro-2',7'-dimethoxy-fluorescein (JOE). Sizing of amplicons is provided by an internal size standard labelled with carboxy-X-rhodamine (CXR). In addition, this screening kit contains *Taq* enzyme and hot-start PCR technology as part of the system.

Sensitivity testing and inhibitor testing data will be shown providing a comparison of this kit with other STR kits. Discussions of laboratory efficiency gains from screening DNA samples with a small STR multiplex will also be included.

**Screening, Forensic DNA, STR**

## A82 High - Throughput Chelex® - 100 Extraction of the Bode Buccal DNA Collector

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After attending this presentation, attendees will understand a new high - throughput technique for processing buccal swabs.

This presentation will impact the forensic community by providing an alternative high - throughput technique for the processing of buccal swabs. This technique does not use robotics, but maintains the minization of human interaction and therefore possible errors.

The primary mission of the Armed Forces DNA Identification Laboratory (AFDIL) is to aid in the identification of missing service members from current and previous military conflicts. To this end, AFDIL works with the Armed Forces Medical Examiner System (AFMES) on current military cases and the Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) for past military conflicts. On average AFDIL receives over one thousand reference samples per year that must be processed accurately and quickly. This presentation will discuss work primarily done by the Mitochondrial DNA (mtDNA) and Laboratory Automation and Biometrics (LABS) Sections in the efforts to process reference samples presumed to be associated with remains recovered by JPAC-CIL.

Bode Buccal DNA Collectors (The Bode Technology Group, Lorton, VA) offer a simple, non-invasive DNA sampling platform, suitable for the collection of reference DNA samples. While it is possible to extract these buccal swabs manually, a 96-well format was desired for a more efficient and time saving technique. Initially, AFDIL used a 96-well format involving DNA IQ™ system (Promega, Madison, WI) coupled with the Biomek® 2000 robotic platform (Beckman-Coulter, Fullerton, CA). Recently, the Biomek® underwent an upgrade to both the heating and the shaker elements, requiring a performance check to confirm that extraction efficiency was not affected by the changes. During this check, it was observed that both blood stain cards and swab extractions failed to produce DNA of suitable quality and quantity for use in nuclear DNA analysis. As extensive additional validation work would be required to return the Biomek® to casework, it was decided to investigate a more rapid and easier alternative for high-throughput extraction of Bode buccal collectors.

AFDIL currently uses an extraction protocol using Chelex®-100 resin (Bio-Rad, Hercules, CA) for manual extraction of DNA from multiple sample types, including oral cotton swabs, Bode buccal swabs, whole blood, bloodstain cards, soft tissue and fresh bone. Incorporating Chelex into a 96-well format offers a rapid, streamlined, and inexpensive solution. Using this system, 90 Bode buccal swabs can be extracted at once, the other six wells being held for extraction and amplification controls. The Bode swabs are punched into a 96-well plate using a Wallac DBS Puncher (PerkinElmer Life and Analytical Sciences, Boston, MA) and submerged in a 5% Chelex®-100 resin solution. Once the plate is sealed, it is placed in a thermal cycler and subjected to a 12.5 hour program, at the completion of which the samples are ready to be amplified for either mitochondrial or nuclear DNA. Multiple plates can be punched and prepared in a single day, the rate limiting steps being how quickly the plates can be prepared and how many thermal cyclers are available for use.

This protocol has proven to be highly efficient and cost effective. Not only does it minimize human interaction and therefore possible errors, it is markedly less expensive than the previously used procedure. Extracting one plate of 90 Bode swab samples with Chelex is approximately 21 times less expensive than the same extraction performed using the DNA IQ™ system on the Biomek®. To date, 1260 mtDNA and approximately 500 nucDNA reference samples have been successfully processed with this technique.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the U.S. Department of Defense.

**DNA, High - Throughput Extractions, Chelex**

## A83 Robotics vs. Organic Extraction for DNA Casework Laboratories

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After attending the presentation, attendees will understand the utilization of robotic platforms in the forensic DNA casework setting in contrast to the more traditional organic extraction.

This presentation will impact the forensic community by illustrating a method to increase productivity while maintaining the current high standards in the DNA field.

In forensic casework laboratories nationwide, DNA backlogs have increased causing proportional demands on the forensic system. The increase in casework volume has a definite strain on the analysts due to the gold standard techniques universally utilized. In order to meet the demands, robotic platforms are entering the DNA casework system in greater numbers. Currently, the gold standard extraction technique, phenol: chloroform with the addition of a filter concentrator, yields a moderately clean extraction product. However, this laborious technique generates hazardous waste and must be preformed in a properly filtered chemical hood. In contrast, robotic platforms can be used in a main laboratory setting while extracting more samples with less manual manipulations.

The evaluation and comparison of the QIASymphony platform and Organic Extraction included commonly encountered forensic sample types, with the addition of the more tedious samples; bones, mixtures, low level sperm and trace. The samples were quantified in triplicate and then processed for capillary electrophoresis. The comparison of the techniques was based not only on speed and precision/accuracy but also on the ability to obtain a profile.

**Robotics, Extraction, QIASymphony**

## A84    The Evaluation of Expert Systems for the Missing Persons Program Using Common STR Kits, Mini-STRs, and Y-STRs

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After attending this presentation, attendees will understand the significance of evaluating expert systems performances when using Y-STR and mini-STR data, especially as it applies to reference sample databasing for the Missing Persons Program.

This presentation will impact the forensic community by providing data from expert systems analyses of Y-STRs and mini-STRs, thereby increasing the scope of the Missing Persons database and the efficiency of the laboratories responsible for processing the reference samples.

In response to the Presidents DNA Initiative, the National Institute of Justice (NIJ) has implemented many programs to increase awareness, provide financial support, and develop new DNA technologies. Additionally, three national laboratories are federally funded through NIJ for the Missing Persons Program: (1) University of North Texas Center for Human Identification, (2) California Department of Justice Jan Bashinski Laboratory, and (3) Federal Bureau of Investigation. These CODIS laboratories use advanced DNA technologies to process unidentified human remains and the family reference samples from biological relatives. The resulting DNA profiles (13 core CODIS loci and/or mitochondrial DNA) are uploaded to the CODIS+mito Missing Persons Index. In this database, mitochondrial and nuclear DNA profiles from the unidentified remains can be searched against the reference profiles; identifications are made through kinship analysis testing. With several hundred-thousand missing persons cases reported each year and more than 14,000 human skeletal remains retained in medical examiners' and coroners' offices, the need for continued support and development of the Missing Persons Program is evident.

Commercially available expert systems decrease sample processing time by automatically interpreting STR data. Due to the excessive number of missing persons cases, there is a potential of twenty-thousand or more reference samples from relatives of the missing to be processed. Expert systems offer improvements in databasing efficiency for reference samples, and it is anticipated that the use of this technology will soon be allowed for uploading family reference samples to the Missing Persons database. It is further anticipated that the CODIS+mito database, which catalogs family references for the Missing Persons Program, will be accepting Y-STR and mini-STR profiles. These additional profiles are of interest because Y-STRs provide information on paternal lineages in males, and mini-STRs may produce additional information in degraded samples. Therefore, this presentation focuses on an evaluation of the performance of three expert systems when using Y-STR and mini-STR data.

The National Institute of Justice's Expert System Testbed (NEST) Project has evaluated several expert systems using NDIS approved STR kits; neither Y-STRs nor mini-STRs have been included in their reports. This study provides a side-by-side evaluation of two expert systems not included in the NEST Project, GeneMarker® HID (SoftGenetics, State College, PA) and FaSTR (Environmental Science and Research,

Wellington, New Zealand). Additionally, GeneMapper® ID v3.2 (Applied Biosystems, Foster City, CA), a software program familiar to the forensic community, is included in the study. The concordance study using these three expert systems will be presented, which includes reference samples amplified with the AmpfℓSTR® Yfiler® and MiniFiler™ PCR Amplification Kits (Applied Biosystems) Samples amplified with AmpfℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) and PowerPlex®16 System (Promega Corporation, Madison, WI) are also included in this study. For each analysis, resulting allele calls and rule firings are evaluated side-by-side for concordance and comparison, respectively. All rule firings are independently investigated and the information is compiled from the cumulative results for each expert system, such as the number of peaks detected and the associated allele calls and rule firings. In addition to the concordance study, the tools, user-interface, and overall functionality of each program are evaluated. Since each expert system is unique, differences in rule firings, tools, and user interfaces are observed and delineated. This information will demonstrate that mini-STRs and Y-STRs can be analyzed using expert systems. Expert systems make accurate allele calls with increased efficiency; this increased efficiency will help Missing Persons Programs address our nation's "silent disaster," the identification of missing persons.

**Expert Systems, Missing Persons Program, Mini - STRs**

## A85    A Tetraplex Real - Time qPCR Assay to Quantify Nuclear and Mitochondrial DNA Determine Sex of the Donor and Detect Inhibition

*Molly McBeth, MFS\*, 1501 Crystal Drive, Apartment 220, Arlington, VA 22202; Christina D. Lindquist, MS, One Shields Avenue, Davis, CA 95616; and Daniele S. Podini, PhD, 2036 H Street, Northwest, Samson Hall, Room 301, Department of Forensic Science, Washington, DC 20052*

After attending this presentation, attendees will learn about a methodology for the simultaneous quantitation of human nuclear DNA and mitochondrial DNA, as well as the determination of the sex of the donor and the detection of inhibition using real-time PCR.

This presentation will impact the forensic community by demonstrating a tetraplex real-time PCR assay capable of quantifying both human nuclear and mitochondrial DNA, determining the sex of the donor, and detecting inhibition in a single 30 minute reaction.

The Quality Assurance Standards set forth by the DNA Advisory Board require the quantitation of human DNA whenever possible. To fulfill this requirement the forensic community has been moving towards a real-time PCR technique. Real-time PCR offers several advantages over other methods of quantitation: primers and probes can be designed to target specific areas in the genome, the assays are sensitive, the method is easy, fast, automatable, and allows for the simultaneous amplification of multiple targets in a sample providing more information from a minimum amount of sample. This assay was designed specifically for the assessment of severely compromised samples. Such samples, due to the small quantity or poor quality of the nuclear DNA, may result in partial or no profiles when analyzed using an STR analysis. However, these samples may contain mitochondrial DNA which could provide information through the analysis of the control region. In order to preserve the sample extract, this assay was developed to quantitate nuclear and mitochondrial DNA in a single reaction allowing for the

immediate determination of whether STR or mtDNA analysis would be most appropriate for a specific sample.

A multiplex real-time PCR assay was developed utilizing the 5' exonuclease detection assay (TaqMan®) using the Cepheid SmartCycler® (Cepheid, Sunnyvale, California) instrument. To quantitate nuclear DNA, mtDNA, detect male DNA and inhibition four primer/probe sets were designed. For the nuclear portion of the assay, the target was a 114 basepair sequence flanking the CSF1PO STR locus. This area contains no known polymorphisms and shares no homologies to other regions of the human genome or to other species. The area targeted for the detection of male DNA was a 111 basepair sequence of the SRY gene on the Y chromosome, downstream from the polymorphic region of the gene. The mitochondrial portion of the assay targeted a 121 basepair area in the mitochondrial 12S gene, between nucleotide 1212 and 1341, which contains few reported rare polymorphic sites (mtDB: http://www.genpat.uu.se/mtDB/). The portion of the assay designed to detect inhibition utilized an internal control DNA that was synthesized to avoid any homologies to any sequence present in nature. Primers and probe were then designed to amplify the 72 basepair synthetic oligonucleotide.

The four probes designed for this assay were each labeled with a different fluorescent dye tag at the 5' end of the oligo: FAM™ for the nuclear probe, TET™ for the male specific probe, TAMRA™ for the mitochondrial probe, and ROX™ for the internal control probe. Appropriate quenchers were added to the 3' end of each probe in order to inhibit fluorescence.

Standard curves were created using 9948 Male DNA (Promega, Madison, Wisconsin). Reproducible DNA concentrations showed a sensitivity of the nuclear DNA to 0.05 ng and of the mitochondrial DNA to 0.50 pg with a 30 minute run time. The detection of inhibition was demonstrated by an increase in the Ct value of the Internal Control in the presence of hematin. The assay was used successfully to determine the appropriate analytical technique for 16 hair samples, three of which were determined to contain sufficient nuclear DNA for STR analysis and the remaining 13 yielded no nuclear DNA but contained sufficient mtDNA for analysis of the control region. The assay provided an accurate means to assess the amount of DNA within a sample. This allows the selection of the most appropriate downstream analytical approach, whether STR or mtDNA, saving time and resources while minimizing sample consumption.

**DNA Quantitation, Real Time PCR, mtDNA**

## A86 Quantitation of Total and Male Chromosomal DNA Using Multiplex PCR and Capillary Electrophoresis

*Robert W. Allen, PhD\*, Department of Forensic Sciences, Center for Health Sciences, Oklahoma State University, 1111 West 17th Street, Tulsa, OK 74107-1898*

After attending this presentation, attendees will have discussed alternative available methods for DNA quantitation using existing instrumentation in the DNA laboratory. Understand the capabilities and limitations of capillary electrophoresis for quantitative measurements. Introduce validation steps for use of Genetic Analyzers for quantitative applications.

This presentation will impact the forensic community by demonstrating the DNA quantation method provides a comparable

alternative to qPCR for quantitating total human and male DNA in a sample. With modest validation of an ABI310 for quantitative use, the assay provides comparable sensitivity and reliability to existing DNA quantitation methods for a fraction of the cost, and with available instrumentation currently in the lab.

The utility of a DNA quantitation assay (Q-TAT) incorporating amplification of the amelogenin gene on the X and Y chromosomes was recently reported (Allen and Fuller 2006). The assay was shown to be comparable to the Quantiblot assay in terms of sensitivity and reliability although it was less sensitive and had a lower dynamic range than qPCR. In this study, the Q-TAT assay has been modified to incorporate additional PCR targets into a multiplex consisting of primers for the amelogenin locus (on both the X and Y chromosomes), the SRY gene (on the Y chromosome), and the luciferase gene from the sea pansy (*Renilla sp*) which was included in the PCR reaction as a template to detect the presence of PCR inhibitors. The enhanced assay (Q-TAT 1.1) was evaluated for accuracy in quantitation of total human and total male DNA, as well as for the detection of known PCR inhibitors. Results showed that the Q-TAT 1.1 assay is reliable and effective at providing accurate estimates of total human DNA. Moreover, in male:female mixtures consisting of as low as 3% male DNA, Q-TAT 1.1 was able to provide quantitation estimates of male DNA suitable for deciding between autosomal STR or Y-STR analyses as the method of choice for DNA typing. Finally, the inhibition control system incorporated into Q-TAT 1.1 is a very sensitive indicator of PCR inhibition caused by EDTA, hemin, indigo dye, and humic acid.

**DNA Quantitation, Capillary Electrophoresis, PCR Multiplex**

## A87 dcDOP-PCR for the Analysis of Compromised Mock and Non - Probative Casework Samples

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The goal of this presentation is to evaluate a newly described low copy number technique ("dcDOP-PCR") using mock and non - probative casework samples like those frequently encountered in the forensic laboratory. Because the recommended input range for commercially available STR multiplex amplification kits is between 0.5 - 2.5 ng of template DNA, there is a need for techniques which can be used to analyze samples that fall below this range and/or samples whose DNA is severely degraded. Previously, this method has been evaluated using serially diluted DNA samples, however, to fully understand the potential of this method for forensic utility, it is important to evaluate mock/non - probative casework samples similar to those frequently encountered in the forensic laboratory.

This presentation will impact the forensic community by proving samples that were pre - amplified using the dcDOP - PCR method had a significantly increased STR allele success rate compared to those amplified with traditional STR procedures (no WGA), producing strong partial or full profiles in many cases where little to no STR data was obtained from traditional STR analysis. Further, STR data quality from

samples pre-amplified with dcDOP-PCR was generally equivalent to or superior to traditional STR analysis. This method could have a significant impact on the forensic community by providing a relatively easy, inexpensive alternative for analyzing compromised and/or low copy number DNA evidence.

The goal of this research project was to evaluate a newly described low copy number technique ("dcDOP-PCR") using mock and non-probative casework samples like those frequently encountered in the forensic laboratory. Although the genetic analysis of DNA has proven to be an invaluable tool in forensic science, it can be problematic when DNA samples are of either low quantity or low quality. Because the recommended input range for commercially available STR multiplex amplification kits is between 0.5-2.5 ng of template DNA, there is a need for techniques which can be used to analyze samples that fall below this range and/or samples whose DNA is severely degraded. One such potential technique, whole genome amplification (WGA) is a method which theoretically preamplifies the whole genome using random or degenerate primers. Studies that have used this approach report that high quality/high yield samples can be obtained from low quantity/low quality samples, increasing the success of downstream applications. It is unknown whether any WGA technique will be beneficial for downstream forensic multiplex STR analysis. Previous reports have described the optimization of one WGA technique, degenerate oligonucleotide primed PCR (DOP-PCR), for use with low copy number, serial-diluted DNA samples. However, in order to fully understand the potential of this method for forensic utility, it is important to evaluate mock/non-probative casework samples similar to those frequently encountered in the forensic laboratory.

Samples evaluated in this study included aged bloodstains exposed to various environmental conditions, cigarette butts, bone, teeth, dermal fingerprints, hair roots, hair shafts, and fired cartridge cases. With the exception of the hairs and cartridge cases, DNA from all samples was initially extracted using the organic extraction method; however, these were later re-extracted using the Qiagen QIAamp® DNA Mini Kit method. Hair roots and hair shafts were extracted using the Qiagen QIAamp® DNA Micro Kit. Cartridge cases were extracted using the Promega DNA IQ™ System. Following extraction, the samples were quantified using the Quantifiler® Human DNA Quantification Kit with the ABI 7500 Real-Time PCR instrument. The samples were then amplified using the dcDOP-PCR method which features a 10N degenerate primer (22-mer, 5'-OH CTCGAGNNNNNNNNNN OH-3', N=A, T, G, C), 12 non-specific DOP-PCR cycles, and *Platinum® Taq*, a *Taq*:proofreading enzyme combination of *Taq:DeepVent*. After Microcon® concentration, the samples were amplified using the AmplℓSTR® Profiler Plus™ PCR Amplification Kit and then post-PCR purified using the Qiagen MinElute® Post-PCR Purification Kit. Finally, the STR fragments were separated on an ABI 3100*Avant* Genetic Analyzer with results analyzed using ABI GeneMapper® ID Software v. 3.2 with a threshold of 75 RFUs. Samples that were pre-amplified using the dcDOP-PCR method had a significantly increased STR allele success rate compared to those amplified with traditional STR procedures (no WGA), producing strong partial or full profiles in many cases where little to no STR data was obtained from traditional STR analysis. Further, STR data quality from samples pre-amplified with dcDOP-PCR was generally equivalent to or superior to traditional STR analysis. Unfortunately, samples extracted organically, particularly those that were environmentally challenged, displayed significant CE artifacts. Thus, it is recommended that this method be used selectively with non-organically extracted DNA samples. This method could have a significant impact on the forensic community by providing a relatively easy, inexpensive alternative for analyzing compromised and/or low copy number DNA evidence.

Low Copy Number, Whole Genome Amplification, Degenerate Oligonucleotide Primed PCR

## A88　High-Performance PCR for Multiplexing STR Loci Directly From Whole Blood

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After attending this presentation, attendees will have learned how a novel PCR master mix can be utilized for multiplexing STR loci directly from whole blood without a need for DNA extraction.

This presentation will impact the forensic community by demonstrating an application made possible by a novel PCR master mix. Whole blood is often used as a source of DNA for human identification and clinical diagnostics. To reduce the manipulations required for analyses, it would be desirable to be able to type the DNA from whole blood without performing an extraction. This has not been previously possible. The practical benefit is that high throughput typing can be facilitated; without a need for extraction, automation can be simplified. In addition, the higher processivity PCR reported provides more consistent yields than with other routinely used methods.

Whole blood is often used as a source of DNA for human identification and clinical diagnostics. To reduce the manipulations required for analyses, it would be desirable to be able to type the DNA from whole blood without performing an extraction. However, *Taq* DNA polymerase, the most commonly used DNA polymerase for the PCR, can be completely inhibited by even small quantities of blood. This inhibition is attributed to the presence of heme. Several strategies have been proffered to overcome this inhibition, such as inclusion of special reaction buffers and additives. Because these approaches have had mixed results, DNA purification and extraction from whole blood still remains a common and necessary practice. In this study, an application of a novel master mix for multiplex PCR amplification and typing of human STR loci directly from whole blood is reported. The master mix contains a *Pyrococcus*-like DNA polymerase that is covalently linked to an Sso7D double-stranded DNA binding protein domain. The Sso7D-DNA polymerase linkage increases the processivity of the polymerase by ~10-fold and makes it extremely tolerant to the PCR inhibitors present in blood. With the master mix, co-amplification of 17 STR loci yields high amplicon yields and full profiles without requiring a DNA extraction step. This approach has been applied to both liquid whole blood and to dried blood stains in which the latter requires only a solubilization step. A PCR can contain up to 10% (by volume) whole blood and yield full profiles with added $MgCl_2$. The benefit of using the Sso7D-*Pyrococcus*-like DNA polymerase is that high throughput typing can be facilitated. Without a need for extraction automation can be simplified. In addition, the higher processivity of Sso7D-*Pyrococcus*-like DNA polymerase provides more consistent yields than with other routinely used DNA polymerases.

**High Performance PCR, Multiplex, Blood**

## A89  A Custom Bioinformatics Tool to Detect Amelogenin Y Allele Dropout in Males

*Jessica Saunier, BS\*, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Rockville, MD 20850; and Michael D. Coble, PhD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, 2nd Floor, Rockville, MD 20850*

The goal of this presentation is to describe the frequency of Y-dropout in a global population database of 1572 samples and to provide a method for the identification of samples with potential Y - dropout. How this process was utilized by the Armed Forces DNA Identification Laboratory (AFDIL) to detect samples and determine the frequency of Y-dropout in its population databases will be demonstrated.

This presentation will impact the forensic community by teaching how to detect samples with potential Y-dropout using a customized macro developed at AFDIL, and will understand the forensic implications of Y-dropout.

Amelogenin is a single copy gene with homologs on the X (AMGX) and Y (AMGY) chromosomes, and is commonly incorporated in commercially available short tandem repeat (STR) kit for human sex identification in DNA databasing and forensic casework. The most commonly used amelogenin primer sets target a region of the first amelogenin intron containing a six base pair (bp) deletion among the AMGX and AMGY alleles.[1] Amplification of both the X (106 bp) and Y (112 bp) alleles indicate a male genotype, while the presence of a single X (106 bp) allele indicates a female genotype. The absence of the 106 bp X-allele does not interfere with gender identification if the 112 Y-allele is present, as the Y-allele indicates the presence of the Y-chromosome.[2] However, absence of the Y-allele, due to mutations and/or deletions in the Y-derived fragment of the amelogenin gene that create an amplification failure, could potentially result in a mistyping of the correct sex.

Frequent Y-dropout has been observed in similar ancestor lineages and in distinct regions of the world, particularly among populations native to the Indian subcontinent [3]. The AFDIL Research Section has previously databased 1572 profiles from Western, Southern, and Central Asian populations using the PowerPlex® 16 System (Promega, Madison, WI). Given the increased frequency of Y-dropout in some of these regional populations, we have investigated the frequency of Y-dropout within our global population databases and have developed a process for the identification of samples with potential Y-dropout.

In order to detect Y-dropout among samples processed in an otherwise streamlined procedure (using electronic data transfer between data reviews and LIMS storage) we have developed a custom macro to flag samples that may have Y-dropout. The macro automatically reads GeneMapper export files and identifies samples with peak imbalance at amelogenin based on the ratio of homozygote and heterozygote RFUs across the entire profile.

The custom designed macro was tested on all 1,572 population samples, and on control samples consisting of known females and males with known Y-dropout. Samples flagged by the macro were subjected to a series of confirmatory tests. All samples were typed with a X-chromosomal STR multiplex which includes SRY, the male sex-determining gene located on the Y-chromosome. In addition, samples were also typed using the AmpFlSTER® Yfiler™ (Applied Biosystems, Foster City, CA) kit which amplifies Y-chromosomal DNA.

Obtaining the frequency of Y-dropout can be important when databasing a large number of samples, particularly when samples are from regions of the world where Y-dropout in the course of standard sample typing occurs more frequently. We will report on the results of our study among 1572 profiles from Western, Southern, and Central Asian populations and discuss this new tool as a method for the identification of Y-dropout.

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**Amelogenin, Y-Dropout, Y-Chromosome**

## A90  Determination of the Sensitivity and Specificity of Six Presumptive Tests for Blood and Their Effect on the Recovery of High Molecular Weight DNA

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The goal of this research was to compare new presumptive tests for blood with each other and with traditional tests. It will show the benefits and drawbacks of the tests depending on their intended use and any subsequent evaluation of the evidence which may be required.

This research will impact the forensic science community by providing an unbiased analysis of the tests. By bringing together the most used presumptive tests and demonstrating their strengths and weaknesses of each, police forces and forensic scientist will be able to make informed decisions of which tests to use under different circumstances.

It is almost never necessary to apply presumptive test reagents directly to dried bloodstain evidence. However, with extremely small samples or when testing large areas it may be necessary to expose potential bloodstains directly to presumptive tests. It is therefore important to know that the tests being used will not destroy the sample for further serological testing. Luminol, leuchomalachite green, phenolphthalein, Hemastix®, Hemident™, and Bluestar© are all used as presumptive tests for blood. In this study, the tests were subjected to dilute blood (from 1:10,000 to 1:10,000,000 dilution factor) to determine the sensitivity of each test. Specificity was determined by reacting the tests with 14 different substances found previously to react with the tests or which could be mistaken for blood. The presumptive tests were applied to blood stains which were subsequently tested for DNA to determine if the presumptive tests damaged or destroyed the DNA.

Five dilutions of blood were prepared using whole blood and sterile water: 1:10,000; 1:100,000; 1:1,000,000; 1:5,000,000 and; 1:10,000,000.

One hundred and fifty repetitions of each dilution of blood were placed on filter paper and allowed to dry prior to analysis. Each dilution of blood was then analysed 25 times with each of the presumptive tests.

Each presumptive test was also analysed 25 times for each of the 14 substances: saliva, semen, potato, tomato, tomato sauce, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1M ascorbic acid, 5% bleach, 10% cupric sulphate, 10% ferric sulphate, and 10% nickel chloride. The effect of each presumptive test on the recovery of DNA was determined by reacting blood with each of the presumptive tests followed by extraction and amplification. The largest and smallest of the SGMPlus™ loci were tested (D2S1338 and D19S433).

All presumptive tests were able to detect blood to a dilution of 1:10,000. Only luminol and Bluestar© were able to detect blood at a dilution of 1:100,000. Phenolphthalein, Hemastix® and Hemident™ did show a reaction at the 1:100,000 dilution level, however the color change reaction took over 1 minute to develop. None of the tests showed a positive reaction with dilutions of 1:1,000,000 or greater. Specificity studies showed that of all the substances tested, not one of the household items reacted with every test, however the chemicals did. Of those substances which did react most could not be mistaken for blood as the reaction proceeded at a different speed and color than what would be expected if blood was present. Results from each test were analysed using a chi-squared test to determine if they came from populations with the same distributions, which would indicate that they react in a similar way.

DNA was recovered and amplified from four of the presumptive tests. Luminol, phenolphthalein, Hemastix® and Bluestar© achieved DNA amplification at both loci tested, which corresponded to the alleles found on the positive control. Hemident™ and leuchomalachite green both produced negative reslts indicating that their use will destroy any DNA present. Phenolphthalein had a much reduced peak height compared with the other three tests indicating inhibition in the PCR or degradation of some of the DNA.

**Presumptive Blood Tests, Sensitivity and Specificity, DNA Recovery**

## A91    A Study of the Environmental Effects on DNA Extracted from Degraded Tissue Samples

*Matt Buettner, BS\*, Florida International University, 11200 Southwest 8th Street, Miami, FL 33199*

After attending this presentation, attendees will see the correlation between degradation of DNA, inhibition of the amplification process, the way these manifest themselves and the method of analysis that provides the most information for sample identification.

This presentation will impact the forensic community by examining the rate of natural degradation of DNA recovered from bodies at crime scenes. This should provide an estimate of time since death for such samples. In addition, by developing a correlation between the quality of real time results and the STR profile, we hope to provide laboratories with the information needed to determine which method of analysis: SNPs, miniSTRs, multiplex STRs etc. will produce the best estimates for sample identification.

The goal of this presentation is to examine the effect of environmental degradation on tissue and bone samples on DNA typed using multiplex PCR analysis. In this project special attention is paid to the connection between measurements of degradation using real-time

PCR quantification and the quality of the recovered profiles for these samples using commercial STR kits.

Using real-time PCR we can generate a virtual yield gel, providing information on the relative amounts of intact and degraded DNA in a sample. This permits the analyst to estimate the potential of generating a full or partial profile using either standard sized or mini STR kits. Ultimately, this should create a more streamlined system in which the relative age of a sample can be determined and the optimal method of analysis can be utilized.

This study involves determination of natural rates of nuclear DNA degradation, under a variety of conditions, to develop a series of timelines for decay. These timelines will then be correlated to the quality of nuclear DNA through the use of real-time PCR utilizing a series of primers varying in amplicon length. These results will then be correlated to the probability of generating a full STR profile, a partial STR profile, or no profile at all. Tissue and blood samples obtained from bodies placed at the University of Tennessee Anthropological Center. Four types of burials were compared: above ground, buried under debris, below ground, and submerged in water.

A series of tissue samples were obtained over weekly intervals and 25 mg of each sample was extracted using the QIAGEN blood and tissue kits and quantified using a series of multilocus Alu based primers with amplicon sizes of 82, 189, and 234 bp respectively. For all three sets of primers, samples 0 to 1 week old yielded DNA recoveries concentrations between 9ng/uL and 12ng/uL. For samples 2 to 4 weeks old, the small and medium primer sets yielded concentrations that would be available for STR analysis while the larger primer set did not. For samples 8 weeks old or older, all three primer sets show concentrations less than 0.05ng/uL, with the majority of samples yielding undetectable amounts of DNA. Samples that could be quantified using real-time PCR were then amplified using PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. Certain samples were also examined using melt curve analysis and other techniques to determine the presence of inhibition. For many of the buried and brush covered samples, inhibition of the amplification process seems to be occurring as seen in melt curve analysis.

The results of this study indicate that samples can be successfully analyzed using real-time PCR to establish the quality of the sample and that these analyses correspond to a particular success rate in the generation of full profiles.

In addition, the data provides information on the relative rates of loss of nuclear DNA in tissue from recovered bodies.

**DNA, Degraded, Environmental**

## A92    Developmental Validation of a Commercially Available Pentaplex MiniSTR Kit

*Melissa Scheible, MFS\*, Rebecca Just, MFS, and Michael D. Coble, PhD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, Rockville, MD 20850*

After attending this presentation, attendees will understand the benefits and limitations of the PowerPlex® S5 System (Promega Corporation, Madison, Wisconsin), a new mini - short tandem repeat (STR) kit that is intended for use as a screening tool for degraded human samples.

This presentation will impact the forensic community by sharing data from the developmental validation of PowerPlex® S5.

Most commercial STR multiplexes contain 10 to 16 loci with amplicon sizes ranging from 100-450 bp. When used to type degraded DNA, allele dropout or the complete loss of the larger loci due to fragmented DNA template is often observed. To utilize the discriminatory power of STR typing with degraded samples, the forensic community has recently focused on the development of "miniSTR" multiplexes: loci with primer binding sites designed closer to repeat regions to reduce their amplicon size. However, to keep the small miniSTR amplicons from overlapping one another, each dye channel can contain only a few loci.[1] The result is a miniSTR multplex with fewer loci than standard STR multiplexes, which in turn leads to a lower power of discrimination. While a relatively low power of discrimination may not be sufficient for identification, small multiplexes can be used as a screening tool for commingled remains. In forensic case work, STR typing with only a small number of alleles recovered has been sufficient to sort the remains of a limited number of known individuals, and STR miniplexes with relatively few loci have also proven successful in resolving large scale circumstances of commingled remains.[2,3]

The PowerPlex® S5 System contains the sex-determining Amelogenin as well as four loci (D18S51, D8S1179, TH01, and FGA) that are included in both the European Network of Forensic Science Institutes (ENFSI) database and the FBI's Combined DNA Index System (CODIS).[4] The marker amplicon sizes, ranging from 90 to 260 base pairs, represent a substantial size reduction for three of the loci (D18S51, TH01, and FGA) when compared to other standard commercial STR kits, making the kit appropriate for use on highly degraded samples. The PowerPlex® S5 System purports to consistently generate full profiles from just 50pg of sample input and is less expensive per sample than other commercial STR kits, and is thus suitable for use as a screening tool in forensic case work. However, the relatively low power of discrimination (random match probability of 1 in 190,000 for U.S. Caucasians[5]) makes the kit less practical for identification and more useful as an exclusionary tool.

Authors will present the results of a concordance study performed with PowerPlex® S5 and three other commercial kits: Powerplex 16®, PowerPlex ES® (Promega Corporation), and AmpFℓSTR® SGM Plus (Applied Biosystems, Foster City, CA)®. Additionally, results from a population study of nearly 500 samples from 5 major U.S. population groups (Caucasian, African-American, "Hispanic", Asian, and Native American) and results of mixture analysis using the PowerPlex® S5 kit will be presented. Lastly, an examination of stutter, precision, and accuracy will be presented to demonstrate the performance of the kit in these respects.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

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**miniSTR, Degraded DNA, Validation**

## A93  The Screening of Buccal Swab Samples With Ninhydrin Solution Results in Improved Cell Collection and STR Success Rates

*Robert Driscoll, MFS\*, Dane T. Plaza, BS, Donia Slack, MS, and Robert A. Bever, PhD, Bode Technology, 10430 Furnace Road, Suite 107 Lorton, VA 22079*

After attending this presentation, attendees will gain an understanding of using ninhydrin solution as an efficient and inexpensive method for the screening and processing of problem buccal swab samples.

This presentation will impact the forensic community by demonstrating an effective method to rapidly screen and locate the highest concentration of buccal cells on buccal collectors.

A method to rapidly screen and locate the highest concentration of buccal cells on a buccal collector by using ninhydrin solutionwill be presented. The occasional failure of buccal swab samples to amplify and generate complete STR profiles is a commonly encountered problem for forensic DNA databanking laboratories. Buccal swab sampling failure can be associated with either the incorrect swabbing of the inner cheek, which results in a non-uniform cell collection and lack of DNA sample, and/or the punching of buccal collectors from areas that do not contain any buccal cells because the areas of high concentration of cells cannot be seen. Both of these failures lead to the need to repeat extraction and amplification processes.

Currently there are no methods in the field that allow for a quick screening process to determine where the highest concentrations of cells are located on a swab. Ninhydrin sprays are commonly utilized in the development of latent fingerprints by detecting the amino acids left on paper substrates. It was theorized that the areas of swiped buccal collectors containing high concentrations of buccal cells and therefore, amino acids, would exhibit darker staining patterns than other areas of the swab when sprayed with this chemical.

Random selections of buccal sample swabs were sprayed with a ninhydrin solution to test for the presence and location of buccal cells on the swabs. A total of 1,425 buccal collectors were tested during this study, and with this new method of ninhydrin screening, the overall first time amplification success rate was improved from 88% to 96%; the second trial amplification rate resulted in 100%. Serial dilution tests of saliva show positive correlations between color intensities and the amount of DNA present on swabs. The darker a swab region stains, the greater the chance there will be substantial amounts of buccal cells/DNA in that area.

These results suggest that the spraying of buccal samples with a ninhydrin solution is an effective, efficient, and inexpensive method for the screening and processing of problem buccal swab samples. In addition, 22 month storage tests have shown no long term destructive consequences from ninhydrin spraying.

**DNA Profiling, Ninhydrin, Databanking**

## A94    DNA From Self - Adhesive Postage Stamps: A Comparison of Four Extraction Methods

*Bonnie S. Stransky, BA*, University of Alabama at Birmingham, 4805 Tree Crossings Parkway, Hoover, AL 35244*

After attending this presentation, attendees will become familiar with four methods for extracting DNA from self - adhesive postage stamps.

This presentation will impact the forensic science community by providing an additional source of DNA that can be collected and used as evidence. The best method for the collection and extraction of DNA from self - adhesive stamps will be described. This research will augment current collection techniques.

Previous works by other researchers have demonstrated DNA profiles can be recovered from saliva residue and transferred buccal cells found on postage stamps and envelope flaps. Due to storage and application convenience, self-adhesive stamps have gradually replaced traditional moisture-activated stamps in the United States. With improving techniques for Low Copy Number (LCN) DNA recovery and amplification, it may be possible to recover full Short Tandem Repeat (STR) loci profiles from epithelial cells deposited on self-adhesive stamps.

The transfer of epithelial cells from an individual to the sticky surface of a self-adhesive stamp provides the opportunity to recover and isolate DNA from self adhesive stamps as a way of identifying the stamp's applier. The extracted DNA must be of a sufficient quantity and quality to allow forensic biologists to amplify STRs, using a commercial kit, producing a full genetic profile. The presence of the stamp's adhesive creates a unique environment as the potential for inhibition from the adhesive must be taken into account. The best method for extracting DNA from self-adhesive stamps is unclear.

Four extraction methods were employed to extract DNA from self-adhesive stamps applied to envelopes by volunteers. Following the application of the stamp to an envelope, the stamps were first cleaned, then removed by peeling the stamp from the envelope. The entire stamp was cut to fit inside a 1.5ml microcentrifuge tube. DNA was extracted from the self-adhesive stamps using one of four methods, an organic extraction with microconcentrators chelex beads, Qiagen's DNeasy Blood and Tissue kit, and Promega's magnetic resin bead DNA IQ kit. Extractions were quantitated and inhibition was evaluated using Appied Biosystems' Quantifiler™ Human Quantification Kit. STR loci will be amplified using Applied Biosystems' AmpfℓSTR® Identifiler® Kit and analyzed using an Applied Biosystems 310 Genetic Analyzer. The profiles generated will be compared to that of reference buccal samples from the volunteers. The extraction method producing the most accurate and robust profiles will be considered the best method. By cleaning the stamps before extraction, an attempt will be made to demonstrate that DNA from the stamp's "user" can be recovered and amplified without amplification of external (postal personnel) DNA.

Initial results indicate DNA can be recovered from the backs of self-adhesive stamps using three of the extraction methods mentioned above. The quantity of DNA should be sufficient to allow for amplification and analysis of STR loci. Conclusions of which extraction method better recovers DNA from self-adhesive stamps cannot be made at this time.

**STR Analysis, Self - Adhesive Stamps, DNA Extraction**

## A95    A Comparison of Collection Methods for Low Copy Number DNA

*Catherine N. Taylor, BS*, 1315 Gary Alan Trace, Moody, AL 35004*

After attending this presentation, attendees will have a basic understanding of different methods commonly used to collect low copy number (LCN) DNA from a non-porous surface. Attendees will understand how the efficiency of five different methods compare to one another.

This presentation will impact the forensic science community by providing information on the relative efficiencies of different LCN DNA collection media. This will be helpful in casework situations where the amount of deposited DNA is minute and the efficient recovery of DNA is crucial. This project may also serve as a framework for future study on the collection of LCN DNA, where additional collection media or surface types may be investigated.

This project explores and compares the efficiencies of different media for collection of low copy number DNA and attempts to ascertain which, if any, of the tested media is likely to recover the largest portion of deposited DNA. The media include cotton swabs, buccal swabs, tape, cotton gauze, and FTA cards.

Full or partial genetic profiles can be obtained from DNA deposited by touch on a surface. A person will shed a variable number of epithelial cells and extracellular material upon coming into contact with the surface. The cellular and extracellular material left on the surface typically contains only a small amount of DNA from the contributor. In order to ensure successful DNA amplification and typing, it is important to collect as much of the deposited DNA as possible. Moistened cotton swabs are generally used for this purpose, but it is of interest whether or not a different medium would consistently collect more DNA. Known amounts of DNA (in the form of an extracellular standard or a known number of cells) were applied to a non-porous surface. Five different collection media were used to collect the deposited DNA: cotton swabs, buccal swabs, tape, cotton gauze, and FTA cards. These collection methods were selected based on their use by other research groups. The collected samples were extracted using an organic extraction method and quantified using real-time PCR.

In order to effectively compare the amounts of DNA collected by the different media, two methods were used in an attempt to deposit known amounts of DNA to a non-porous surface prior to collection. In the first set of experiments, a known volume of an extracellular DNA standard was deposited on the non-porous surface. In the second set of experiments, a method was developed for depositing a known amount of buccal cells to the non-porous surface. This was done by diluting a solution of buccal cells until 7 uL of the solution, applied to a glass microscope slide, would contain a number of cells that could be counted when viewed under the microscope. The cells were stained and counted using a compound microscope. The cells were then collected from the slide with one of the five collection media and DNA was extracted using an organic extraction method.

**Low Copy Number (LCN), DNA Collection, DNA Quantitation**

## A96 High–Throughput Processing of Mitochondrial DNA Analysis Using Robotics

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The goal of this presentation is to inform the forensic community as to the uses and applications of robotics for high - throughput processing of DNA samples for mitochondrial DNA testing.

This presentation will impact the forensic community by offering alternatives to sample processing through robotics in order to increase efficiency and sample throughput, resulting in additional time for analysts to perform more complex tasks and analysis.

The adoption of multi-capillary instrumentation in the forensic community has resulted in the transition from single tube testing to a 96-well plate format as seen in real-time PCR, thermal cycler, and genetic analyzer sample layouts. The identifications of thousands of deceased individuals from the World Trade Center terrorist attack on September 11, 2001 have launched the use of robotics to processing samples using these 96-well plate formats. The data generated using automated systems suggests that robotics is amenable to forensic applications.

Several companies have generated robotic systems that are capable of automating key steps in processing forensic samples. Robots can be configured with fixed single and multiple pipetting tips or disposible tips, and accessorized with shakers, heaters, cooling systems, vacuums, grippers, and plate stackers. In addition, robots can be coupled to quantification equipment such as a luminometer or fluorometer, as well as thermal cyclers used for amplification and sequencing. There are limitations and advantages for every robot. Many laboratories face financial constraints, and are unable to purchase high end robots. Fixed format robots are typically more affordable than robotics amenable to multiple accessories. Because the features of each robot are different, automation script designs must be written to accommodate the robot used for its specific application.

An ideal forensic pre-PCR robotic system could incorporate DNA extraction, quantification and normalization of DNA, and PCR reaction setup, all within a 96-well plate layout. Preferred post-PCR robotics would consist of PCR quantification and normalization, product purification, and preparation of sequencing reactions. The University of North Texas Center for Human Identification houses a Freedom EVO® 100 (Tecan Group Ltd., Männedorf, Switzerland) and two MiniPrep 75 Sample Processors (Tecan Group Ltd.). The Freedom EVO® 100 is used for extraction of reference DNA samples associated with the Missing Persons Program, using the DNA IQ™ System (Promega Corporation, Madison, Wisconsin). We use a modified script design to decrease the number of plate transfers and introduce bleach washes. It was necessary to introduce the bleach washes since the extracted DNA was being used for not only STR, but also the highly sensitive mitochondrial DNA (mtDNA) testing.

Scripts for two MiniPrep 75 Sample Processors (MiniPrep) have recently been developed, which are fixed liquid handling format robots, for the amplification, post-amplification clean-up, and cycle sequencing reactions for the high-throughput sample processing of mtDNA samples. Each MiniPrep is housed in a specific laboratory, either pre-PCR or post-PCR. Robotic automation scripts for the MiniPrep were designed for amplifying the hypervariable regions (i.e., HV1 and HV2) of mtDNA

utilized in forensic sciences. The 96-well template DNA plate is placed onto the pre-PCR MiniPrep, along with two empty 96-well plates for reaction setup. The script was designed to aliquot the HV1 and HV2 master mixes into the respective 96-well plates. Template DNA is then transferred to each of the plates containing master mixes. Upon completion, the HV1 and HV2 plates are sealed and placed on the thermal cycler for amplification.

Three separate scripts were created for the post-PCR MiniPrep. The first script was designed to perform enzymatic post-amplification cleanup using ExoSAP-IT® (USB Corp., Cleveland, OH). The two 96-well plates containing PCR products are added to the MiniPrep. The single-tip pipette transfers ExoSAP-IT® from a tube into a clean 96-well plate. The fixed 8-tip pipette then transfers the ExoSAP-IT® into the plate containing the PCR product, introducing a bleach wash before each addition. The sample plates are then sealed and placed on the thermal cycler. A second post-PCR script performs cycle sequencing setup using the ABI Prism® BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The plates of purified PCR product are placed on the MiniPrep. Sequencing master mix is aliquoted into each well of a new 96-well plate using the single-fixed pipette tip. Purified PCR product is then transferred to the respective plates. Plates are then sealed and placed on the thermal cycler. The third script performs sequence cleanup using Edge Performa® Plates (Edge Biosystems, Gaithersburg, MD). The plates of cycle-sequenced product are placed on the MiniPrep. The 8-tip arm then transfers the entire sample to the column plate. The plates are then removed from the MiniPrep and centrifuged and the purified product retained. The plates are ready to be directly placed on the genetic analyzer for capillary electrophoresis.

This research has shown that successful amplification can be set up twith ExoSAP-IT® purification cycle sequencing reactions, and post-sequencing clean-up on the MiniPrep 75 Sample Processor robot. Sequence data obtained displays minimal background noise, and to date, no contamination has been detected from any of our samples. Automated robotic systems can reliably be used to process forensic reference samples for sequencing.

**Robotics, Automation, Mitochondrial DNA**

## A97 Evaluation of Audit Trails and Security Features in Software Systems

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After attending this presentation, attendees will acquire a broad knowledge of the different audit and security features offered in a variety of software programs. Participants can use this knowledge base in helping them choose a software system for their laboratory.

This presentation will impact the forensic community by increasing awareness of the audit trails and security features available in different software systems.

The use of software systems in forensic DNA testing has played an integral role for years in DNA analysis, even with RFLP sizing. And even more recently, LIMS and expert systems are being validated and adopted

into the workflow of forensic DNA laboratories. With the increase in electronic automation, the advances in paperless systems, and the development of software tools, sophisticated algorithms and audit trails are being introduced. As DNA data analysis becomes more automated in processing, the evaluation of proper documentation and user tracking is essential. In court, it is crucial when an analyst makes a change to an allele call, that it be traced to the analyst who made it. When looking at software systems, security features such as analyst login, administrative control, and audit trails should also be evaluated to ensure they meet the laboratory's quality assurance requirements.

The NIJ Expert System Testbed (NEST) Project Team has evaluated several single source expert systems and mixture deconvolution tools including: DNA_Data Analysis Software (United States Army Criminal Investigative Laboratory, Fort Gillem, Georgia); FSS-i3™ Expert Systems Software version 4.1.3 (Promega Corporation, Madison, Wisconsin) in conjunction with GeneMapper® *ID* Software version 3.2 (Applied Biosystems, Foster City, California); GeneMapperâ *ID* Software version 3.2 (Applied Biosystems); GeneMapper® *ID-X* Software (Applied Biosystems); TrueAlleleâ Databank version 2.9 (Cybergenetics, Pittsburgh, Pennsylvania); and TrueAllele® Casework System Package (Cybergenetics). All of these software systems offer different tracking and administrative control features. When launching the software, some systems have unique user login and password requirements, while other software programs require entering a user ID at an alternate point during sample analysis. There are also software programs that track the Windows user login, and associates that user with a data set imported into the software. The number of software licenses purchased may dictate which type of user tracking best fits the laboratory.

Audit trails document the user logged in, changes made by the user, and the rules that fired, to name just a few examples. Each software package reports different information in its respective audit trails. Another level of security examined is the accessibility of settings to various users. There are software packages that allow the administrator to limit access to custom settings, and some that do not. For example, if the laboratory's quality assurance program states that only an administrator or a technical leader can modify settings and thresholds, access to these settings can be controlled in some software packages.

The intent of this presentation is to inform the forensic community of the differences in security and audit features in single source expert systems and mixture deconvolution tools. The information presented in this poster may assist a laboratory in choosing not only a software program that meets both its analytical needs but its security needs as well. Comparisons between the software packages will be discussed, highlighting the benefits as well as possible areas for improvement for each program assessed.

**Expert Systems, Audit, Security**

## A98    Comparison of a Commercial mtDNA Sequencing Kit to Standard mtDNA Sequencing Protocols

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After attending this presentation, attendees will have gained knowledge of the typical sequence coverage, cost, and ease of use of a commercial mtDNA sequencing kit as compared to the standard sequencing protocol used for mitochondrial DNA (mtDNA) population databasing at the Armed Forces DNA Identification Laboratory (AFDIL) in Rockville, MD.

This presentation will impact the forensic community by providing information relevant to practical considerations for the implementation of mtDNA sequencing in forensic laboratories.

Over the past eight years, the Armed Forces DNA Identification Laboratory has sequenced greater than 15,000 entire mtDNA control regions (CRs) and 500 entire mtDNA genomes as a part of the laboratory's grant-funded databasing projects. Sample processing typically utilizes protocols optimized for high quality samples, and is performed in a high-throughput, 96-well format from extraction through sequence detection. Robust and reliable sequence data is assured by redundant sequence coverage, particularly in regions prone to length heteroplasmy (LHP), and through additional safeguards in data analysis and data transfer.[1, 2]

New commercial mtDNA sequencing kits for the mtDNA CR and entire mitochondrial genome are marketed as an out-of-box alternative to standard sequencing methods for the detection of sequence variation. In contrast to most PCR-based methods which use a large number of sequencing primers to ensure complete sequence coverage, these commercial kits instead utilize a greater than usual number of amplicons along with M13 primers for universal sequencing.

A comparison of the commercial mtDNA CR and entire mitochondrial genome sequencing kits to AFDIL's standard mtDNA sequencing protocols for population databasing will be reported. The sequence coverage obtained by each method on replicates of positive control DNA and on high-quality population samples, particularly in the hypervariable regions (HV1, HV2, and HV3) of the mtDNA CR was investigated. The AFDIL and commercial sequencing protocols use a similar number of sequences to cover the mtDNA CR (16-17 and 18 sequences, respectively), and a similar percentage of nucleotide bases with double-stranded sequence coverage was obtained using the two protocols. However, the results of the study indicate that significantly greater re-processing would be required in order to obtain complete double-stranded coverage with the commercial CR kit as compared to the AFDIL protocol. This result was due in part to sequence coverage differences between the kits in regions where LHP occurs frequently. When LHP is encountered in a sample, any additional sequence failures or poor quality sequence data can easily result in gaps in double-stranded coverage if there is insufficient redundancy in the sequencing strategy. Given that approximately 50% of all individuals will have at least one LHP in the CR,[3] frequent gaps in double-stranded coverage when LHP is encountered may substantially increase sample processing and analysis time and costs for the commercial kit relative to the AFDIL protocol.

These comparisons of sequence coverage, as well as considerations related to the ease of laboratory processing and sample processing costs for both methods, will be presented.

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**Mitochondrial DNA, Sequencing, Databasing**

## A99    Effectiveness of Three RNA Extraction Methods for Body Fluid Stains

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After attending this presentation, attendees will gain a better understanding of messenger RNA (mRNA) profiling as it pertains to forensic samples, the complexity involved in adapting this technique for forensic samples, and various methods which may be employed to extract RNA from forensic body fluid stains and evidence.

This presentation will impact the forensic community by demonstrating the application of different RNA extraction methods to forensic samples. Additional benefits to the community include decreasing RNA extraction time, decreasing forensic sample consumption, as well as allowing for the possible implementation of robotics for sample processing.

This study was initiated to compare and contrast two common RNA extraction methods (an alternate organic extraction method and a silica filter based method) to the current (phenol: chloroform based) standard operating procedure (SOP), for their ability to extract RNA from body fluid stains. The primary objective was to demonstrate that methods exist that with modification, can be used to extract RNA from forensic samples with more efficiency than the current SOP.

Forensic analysis of body fluid stains traditionally involves serological analysis. In recent years a novel molecular technique, mRNA profiling, has emerged as a possible alternative to serological methods. Several different methods can be employed to extract RNA, although typically the protocols are designed for extraction from tissues or cell lines directly rather than forensic body fluid stains. Ballantyne et al have proposed a SOP for RNA extraction from body fluid stains based on a standard phenol: chloroform extraction and this method was utilized for comparison in these experiments. However, this method is tedious and time consuming. The TRIzol method (Invitrogen) was chosen because it utilizes phenol: chloroform as the primary mode of action for the RNA extraction, while the RNAqueous® -4PCR kit (Ambion) was chosen to represent the popular silica filter based extraction method. For both methods it was necessary to modify and optimize the protocol to accommodate forensic body fluid stains. The modifications primarily

involved adding a step to denature and lyse the RNA from the body fluid stains prior to applying the sample to the extraction method. All RNA extraction methods were completed by multiple users to verify the robustness of the method. It was found that the SOP requires a greater degree of training and skill than either the TRIzol method or the RNAqueous® -4PCR kit. Saliva stains allowed to dry overnight on sterile cotton swabs were used for initial comparisons between the methods. When comparing the two phenol: chloroform extraction methods, the results demonstrated that despite the TRIzol method being faster, the SOP method yielded an average of 6.5ng/μL more RNA per sample. The silica filter based RNAqueous® -4PCR extraction method is also much faster than the SOP. A comparison of this method with the SOP shows that a comparable amount of RNA can be extracted. Thus, it can be concluded that with modification, commercially available methods have the potential to be utilized to extract RNA from forensic stains in a more efficient and user friendly manner.

**mRNA Profiling, Body Fluid Stains, Extraction**

## A100    Towards a Microfluidic Device for Integrated Purification and Amplification of RNA

*Kristin A. Hagan, BS\*, Alison H. Dewald, MEd, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will gain an understanding of the development and applications of a microdevice that can process a biological sample by first isolating and purifying RNA followed by RT - PCR amplification.

This presentation will impact the forensic community by introducing a microdevice for integrated purification of RNA and RT-PCR analysis for forensic body fluid identification. This device will allow for faster evidential analysis and increased throughput for forensic casework sample processing. The methodology greatly reduces the number of sample transfer steps and is performed in the closed environment of a microfluidic device, reducing the potential for contaminants and RNases to enter the system and degrade a sample. This work is another step towards the realization of a micro-total analysis system for body fluid identification using mRNA expression analysis. The purpose of this research is to demonstrate the development of a single microfluidic device for the integrated solid phase purification and subsequent RT-PCR amplification of RNA for forensic body fluid identification.

mRNA expression analysis is based on inherently variable mRNA expression from different cell types, producing gene-specific patterns[1] which can verify the presence of individual body fluids present in a forensic sample.[2] The importance of body fluid identification in an investigation is realized when it is necessary to determine the number of contributors in a sample, as well as when a body fluid, in the form of a stain, originates from a single person. The origin of each body fluid can be determined, providing important information to investigators clarifying the criminal act which took place.

Prior to analysis, RNA from a biological sample must be isolated. Reverse transcription-PCR amplification and separation of target amplicon can then be performed to identify the sample. Conventional methods for RNA extraction do not guard against the inherent sensitivity to degradation and contamination of RNA because they involve many transfer steps exposing the sample to exogenous contaminants and

RNases. The use of microfluidic purification systems for DNA has been well-characterized as an alternative to conventional DNA isolation, allowing for reduced sample and reagent consumption, as well as a reduction in analysis time.[3, 4] This technology has also been applied and proven sound for the isolation of RNA from biological samples, providing purified, amplifiable RNA as a result.[5]

In addition to solid phase extraction (SPE), PCR amplification on a microdevice has also been demonstrated, with advantages including smaller reaction volumes (and therefore reduced reagent consumption), possible integration with up and down stream applications, and reduced analysis times.[4, 6] Specifically, the smaller thermal mass associated with microfluidic PCR enables sharp temperature transitions, decreasing not only the time needed for amplification, but also the formation of nonspecific product.[7] Integrated on-chip solid phase extraction (SPE) and PCR for DNA has been shown previously by our group, using infrared-mediated (IR-mediated) heating.[6] Incorporating microchip-based PCR into a system for SPE-RT-PCR for mRNA analysis utilizing IR-mediated heating would greatly benefit the forensic community by providing a closed-system platform for RNA isolation and amplification, reducing sample transfer steps and, therefore, reducing contact of the sample with exogenous contaminants and RNases.

This work proposes to be the first demonstration of a microfluidic system for the silica phase-based purification of RNA integrated with RT-PCR using IR-mediated heating, to reduce thermocycling times, while demonstrating applicability of the system to forensic samples. By integrating the IR heating method for faster RT-PCR with solid phase extraction of RNA, total analysis time would be greatly reduced, making identification of body fluids by mRNA expression more applicable to time-sensitive analyses. Microchip-based SPE (μSPE) will be integrated with microchip-based RT-PCR, utilizing non-contact IR-mediated heating for PCR amplification, bringing the forensic community a step closer towards an integrated micro-total analysis system capable of total mRNA profiling. Experiments detailing studies for optimization of time necessary for reverse transcription as well as cycling times for PCR on a microdevice are included. Also, work towards the extraction of RNA on a microdevice followed by microchip-based RT-PCR amplification will be shown.

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**RT - PCR, Microfluidics, Purification**

## A101  Significance and Reliability of Presumptive Testing for Semen

*Belinda M. Potter, MFS\*, Kansas City Police Crime Laboratory, 6633 Troost, Kansas City, MO 64131*

After attending this presentation, attendees will understand the experimental differences between the AP spot test, the PAN test, and the diSTMP test and their effectiveness, based on Bayesian statistical theory. Attendees will also be informed as to the effectiveness of the presumptive semen test diSTMP over a period of six years and the apparent differences between the analysis of a specific type of sample and the amount of DNA ultimately recovered.

This presentation will impact the forensic community by serving as an example of a long - term study in the types of samples and the DNA that is recovered. This could impact how fellow laboratories analyze samples and which presumptive semen test they use. The experimental portion of the study also gives the community an opportunity to objectively assess three presumptive semen tests. The use of Bayesian statistics allows for a completely objective methodology to analyze the effectiveness of tests and this study gives an example of how this statistical analysis can be used.

By using a combination of a statistical analysis and an experimental study, this project strived to give a more objective answer as to which presumptive test for semen would be most effective for the Kansas City Police Crime Laboratory (KCPCL).

The statistical portion of the project took six years worth of casework and correlated the results of diSTMP AP testing and whether a foreign DNA profile was present. The results of this portion of the study directly impacted how KCPCL analyzes evidence and has made the laboratory aware of the inherent differences in the types of samples commonly encountered. This portion of the project showed that, generally, stains elicit a foreign profile more often than swabs. Non-cavity swabs are responsible for the highest amount of false negatives, while cavity swabs have the highest rate of false positives. Overall, the diSTMP test correctly identified the presence or lack of semen 70% of the time, with 26% of false positives and 4% of false negatives. The laboratory continues to gather the same type of statistical information to use in future evaluations of the new presumptive semen test.

In the experimental portion of the study, three common presumptive semen tests were compared with known samples to objectively assess their specificity and sensitivity in analyzing these samples, specifically using Bayesian statistical theory. The three tests were the diSTMP test, the AP Spot test and the PAN test. Based on the results, the AP spot test was found to be more effective than the diSTMP test, although the PAN test proved to be the better test. The difficulty in interpreting the PAN test made it an unrealistic choice for KCPCL. Currently, the laboratory has validated and is using the AP spot test in casework.

Additionally, KCPCL has also implemented a semen analysis scheme based on the results of this study. At the conclusion of this study, the marked difference between stains and swabs was obvious and a new semen analysis scheme was realized. Stains are treated first to the AP spot test, while swabs are screened with the p30 test (PSA Semiquant card). This updated analysis scheme allows for the weaknesses of both types of test to be strengthened based on the sample being examined and also gives examiners an improved sense of confidence in the presumptive testing for semen.

**Semen, DNA, Acid Phosphatase**

## A102 Rapid IR and Gel Electrophoresis "Fingerprinting" Methods for Characterizing Body Samples Including Blood, Saliva, and Hair Evidence

*Kelly M. Elkins, PhD*, Metropolitan State College of Denver, Chemistry Department, PO Box 17362, CB 52, Denver, CO 80217*

After attending this presentation, attendees will gain an appreciation for methods that differentiate between body samples that may contain blood, saliva, and hair using attenuated total reflectance infrared spectroscopy and agarose gel electrophoresis. In particular, each one of these three evidence samples and their components exhibit different absorption frequencies and intensities and electrophoretic mobility. Attendees will also learn how the use of appropriate reference standards can assist in spectral and gel interpretation, especially in the case of complex mixtures.

This presentation will impact the forensic science community by establishing the scientific basis for direct spectroscopic and electrophoretic methods of qualitatively identifying evidence at a crime scene. Both methods allow an investigator to determine the presence of biological samples at a crime scene in an expeditious manner using non-destructive techniques. The determination of having saliva or blood or both present may help the investigator to decide how to collect and package the sample for further DNA processing. Rapid tools for differentiating body samples and non-biological samples such as paint might also help investigators determine the relevance of an item of trace evidence. Electrophoresis has found utility in species determination.

Methods to replace the currently used presumptive test reagents for blood (luminol, phenolphthalein, and leucomalachite green) and saliva (Phadebas tablets) are continuously being pursued. These tests often render a sample unusable for genetic testing. FT-IR has been employed to examine the hemoglobin component of blood (Dong and Caughey, 1994, Gregoriou et al., 1995) and ATR FT-IR was recently employed to analyze hair morphology (Lyman and Schofield, 2008). Although FT-IR spectroscopy has not been used directly to analyze saliva or amylase, it has been used in characterizing amylase activity in digesting starch, amylose and amylopectin by analyzing the degradation of the original substrate molecule (Marcazzan et al., 1999). IR has also been used to measure the adsorption of salivary proteins onto soft denture lining materials (Imai and Tamaki, 1999). Protein electrophoresis is used to evaluate, diagnose, and monitor a variety of diseases and conditions. It is used clinically to separate and evaluate the albumin, $\alpha1$, $\alpha2$, $\beta$, and $\gamma$ proteins and to evaluate blood disease by differentiating between the different types of hemoglobin in the blood including A, F, S, C, E, D. H. Kelly et al. (1991) used gel electrophoresis to perform studies of human apolipoprotein components of blood from whole blood and blood stains. Gel electrophoresis is a simple, rapid, reliable, and economical technique that allows for the simultaneous analysis ("fingerprinting") of a variety of polymorphic enzymes and proteins and allows for the estimation of nano- and picogram quantities of enzymes and proteins. This technique has been used to differentiate blood samples and to attribute them to either brown or black bear species. It can also date the time elapsed since the sample was deposited (Wolfe, 1983). Electrophoresis and isoelectric focusing have been used to separate and to identify proteins from blood (Grunbaum, 1977), hair (Lee et al., 1978; Folin and Cau, 1990; Khawar et al., 1995) and saliva (Tenjo et al., 1993; Yasuda et al., 1996). SDS-PAGE and immunoblotting was used to detect ABH blood group antigens in saliva and their stability according to storage of saliva samples (Kim,

2003). Human hair keratin was analyzed by electrophoresis to obtain specific-species patterns (Folin and Contiero, 1996) and the human hair proteome was recently analyzed by LC-MS and LC-MS/MS (Lee et al., 2006). Tie et al. (1993) used capillary electrophoresis to characterize human seminal plasma.

The research described in this presentation utilizes the entire IR spectrum including the amide banding regions and the fingerprint region of the spectrum. Although the FT-IR spectra of blood dried at three temperatures (21ºC, 40ºC, 120ºC) to simulate conditions of arson or fire do not differ in the frequencies of the exhibited peaks, these spectra can reveal a complex mixture relative to a hemoglobin standard. All spectra obtained were of solid samples which demonstrate a major advantage to the use of ATR FT-IR spectroscopy: it is fast, reliable, and requires little or no sample preparation. FT-IR absorbance spectra (32 scans, 4000 to 400 $cm^{-1}$ spectral range, 1.929 $cm^{-1}$ spectral resolution) using the ATR diamond crystal in ambient temperature as a background, IR spectral data sets were recorded for simulated forensic samples (dried blood at varying temperatures, dried saliva, and shed hair) and comparison reference samples of known components (hemoglobin, albumin, amylase, free amino acids). Finally, the spectral regions most relevant to the differentiation of the biological samples were identified. Predictive accuracy appears to be independent of temperature. In the electrophoresis experiments, the components of the complex mixtures of blood and saliva separated and provide an informative fingerprint for these substances as the components vary in size and charge and migrate at different rates. Agarose gel electrophoresis was used because it is non-toxic and sets up and runs more quickly than polyacrylamide gels. Both gels can be commercially purchased pre-made. 0.8% gels were employed to reduce the cost of the analysis. Coomassie blue stain was used to visualize the bands. The use of higher percentage gels reduces discriminating power in the short time frame (30 minutes maximum) used. Electrophoresis is also non-destructive, requires only nanogram or even pictogram quantities of sample, and allows the sample to be recovered from the gel material. Hemoglobin, albumin, amylase reference standards were used to verify the protein composition of the samples. This research could be extended to include qualitative examinations of urine, feces and semen using these methods.

**ATR FT-IR, Agarose Gel Electrophoresis, Biological Evidence**

## A103 Effect of Hydrogen Peroxide Pre-Treatment on the Results of Subsequent Phenolphthalein Presumptive Test for Blood

*Anna Timanova, PhD*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will learn that pretreatment with hydrogen peroxide greatly inhibits subsequent phenolphthalein testing producing a false negative reaction. The audience will be led through the experimental strategy leading to the reported results and an explanation of why subjecting a sample to more then one presumptive test for blood should be approached with care.

This presentation will impact the forensic community by demonstrating a possible interaction between the presumptive tests for blood and will help analysts select a proper approach when more than one presumptive test needs to be performed.

Agencies have adopted different presumptive tests for blood in their standard procedures. It is not uncommon for more than one presumptive

test to be performed in situations when an evidence item is analyzed by more than one agency. Each agency will attempt to identify the presumptive presence of a substance according to their own procedure. This raises the possibility of interference or incompatibility of the presumptive tests. Most of the presumptive tests for blood are based on the peroxidase-like catalytic activity of hemoglobin and the use hydrogen peroxide as an oxidant. Pretreatment with hydrogen peroxide is standard practice in immunohistochemistry when blocking the endogenous peroxidase activity of the specimen is desired.

Therefore, a study was carried out to assess the potential effect of pretreatment with hydrogen peroxide on the sensitivity of a subsequently performed phenolphthalein test, routinely used as a presumptive test for blood in the Harris County Medical Examiner's Office Forensic Biology Laboratory.

The results demonstrated that pretreatment with hydrogen peroxide greatly inhibited subsequent phenolphthalein testing producing a false negative reaction. The likely mechanism is the inhibition of the endogenous peroxidase activity of hemoglobin. Since most of the presumptive tests for blood rely on the hemoglobin endogenous peroxidase activity and use hydrogen peroxide as an oxidant, care should be taken when subjecting a sample to more then one presumptive test.

An example from casework where samples were treated with fluorescein prior to submission to the laboratory is presented. Results of presumptive, confirmatory, and DNA testing will be presented. Additional studies on the effect of sample pre-treatment with hydrogen peroxide and/or phenolphthalein testing on subsequent confirmatory testing such as ABAcard® Hematrace® will also be discussed.

**Presumptive, Fluorescein, Phenolphthalien**

## A104  Concealment and Detection of Bloodstains Beneath Multiple Coats of Paint

*Jackie A. Clanton, BA, 1545 South 13th Street, Lincoln, NE 68502; and Melissa A. Connor, PhD\*, 11101 South 98th Street, Lincoln, NE 68526*

After attending this presentation, attendees will learn how different types of paint cover bloodstains and how Fluorescin with the use of an alternate light source, helps to detect bloodstains when they are no longer visible to the naked eye.

This presentation will impact the forensic community by providing information on ways bloodstains can be detected under multiple coats of paint.

This experiment used two popular brands of paint, Lucite Satin Finish latex paint and Zinssers Bulls Eye 1-2-3 Primer a product specifically formulated to hide and seal off stains. Both paints were a basic white and were applied with a standard compressed air paint spray rig. The blood spatter was applied using an Iwata airbrush rig and a paint scraper to deflect the blood and create the spatter effect.

One foot square pieces of untreated drywall were used for the experiment. Five squares were used as controls. The negative controls were treated as follows: one was left untreated, one was painted with basic primer, one was painted with one coat of basic primer and two coats of paint, and one was painted with a single layer of Zinssers. The positive control received one coat of the basic primer, two coats of paint and one coat of blood spatter.

Twenty-four squares were divided into two groups of twelve. Group A received the basic primer coat, two coats of paint, and bovine blood spattered on each square. Once the blood spatter dried, each square received a coat of paint; then additional coats of paint were applied. Square A1 got one coat of paint over the blood spatter. A2 got two coats of paint over the blood spatter and so on up to A12 which received twelve coats of paint over the blood spatter. All paint coats were allowed to dry at least 24 hours before the next coat was applied.

The second twelve drywall squares, Group B, were painted with the primer, two coats of paint, then bovine blood was spattered on the drywall, and then one coat of Zinssers applied over the blood before the consecutive coats of paint were applied. Drywall squares B1 through B12were painted with increasing numbers of coats of paint in a manner similar to the drywall squares in the "A" sequence.

All paint and Zinssers coats applied after the blood layer were measured at exactly 40 milliliters per coat. The blood spatter was created with 2 milliliters of bovine blood. The bloodstains were no longer visible to the naked eye on drywall squares containing more than two coats of paint in both groups.

The squares were examined with an alternate light source set on 415 nanometers and crime scene setting with a yellow filter on the camera, before the application of Fluorescin. The bloodstains were visible under the satin finish paint up to four coats deep and under the Zinssers 1-2-3 Primer up to seven coats deep. After the application of Fluorescin, the squares were examined with an alternate light source set at 490 nanometers and white with an orange filter. The bloodstains were visible under all twelve coats of paint in both groups. Orange goggles were used for eye protection for viewing with the alternate light source before and after the application of Fluorescin.

During the application of Zinssers onto the twelve squares in Group B, the completely dried blood seemed to bleed. Upon closer inspection it was discovered that the Zinssers had actually separated from the blood leaving a pocked-marked surface visible under several coats of paint. The variable surface could be seen with the naked eye when holding the squares under certain lighting conditions.

**Bloodstain, Fluorescin, Paint**

## A105  Statistical Methods for Reducing Inaccurate Bias During Manipulation of Data Below the Detection Limits in Forensic Investigation

*Robert Posey, MChem\*, University of East Anglia, Earlham Road, Norwich, NR4 7TJ, UNITED KINGDOM; and Jurian A. Hoogewerff, PhD, Centre for Forensic Provenanging, School of Chemical Sciences, University of East Anglia, Norwich, Norfolk NR4 7UA, UNITED KINGDOM*

After attending this presentation, attendees will be introduced to the risk of data set biasing by incorrect treatment of results below the experimental detection limits (censored data) and will leave with knowledge of a number of data handling techniques suggested in a number of environmental sources for the treatment of such data. Attendees will also be introduced to some powerful statistical software to facilitate the use of these techniques.

This presentation will impact the forensic community to the need for more universal methods for the analysis of non-detect data in order to prevent ambiguity between groups, and to reduce the risk of biasing forensic data sets which could lead to inaccurate conclusions. It will introduce techniques available for the analysis of non - detect data that can result in more accurate conclusions that the commonly used detection limit substitution methods.

Forensic investigation often involves the analysis of materials containing only trace levels of the analyte of interest. Data sets from such investigations often contain values that lie below the detection limit of the analytical method. Traditional methods for dealing with these values involve either substitution of a value as a function of the detection limit, (essentially fabricating the data based on no theoretical data), or by removing the value altogether. These techniques can significantly bias the summary data for a data set due to the population distribution becoming skewed. A number of statistical techniques have been proposed by the environmental community for the calculation of summary data for data sets containing censored data values based on the population distribution of values within the data set above the detection limits. There are also methods proposed for the calculation of summary data for data sets containing only censored data. Of particular interest are the methods described by D.R. Helsel (2005) which can easily be performed using the NADA for R macro in the powerful statistical software R.

The author will present results for the concentrations of trace elements in a selection of 104 European olive oils from 5 countries, which were measured using a novel LA-ICP-MS technique developed at UWA funded by the European Commission TRACE project. Olive oils are notorious for containing very low levels of trace elements (ppb) and the novel laser ablation technique was proposed as a possible improvement on traditional sample dilution techniques. A number of groups have reported successful differentiation of olive oils from different regions but only on very small sample sets and a limited number of elements suggesting stochastic difference causing the differentiation rather then a deterministic process. Our sampling scheme should therefore represent a more realistic approach. As expected a high percentage of the data was censored. Summary data was obtained for 25 elements using non-detect analysis techniques. Between group significance testing was performed using Peto-Prentice, MLE and ANOVA methods and concluded that no differentiation could be made between the geographical regions using the data available. Analysis was made possible by the implementation of the non detect analysis techniques without which little conclusion could be made from a data set with such a high percentage of censored values.

**Non-Detect, Statistics, Bias**

## A106 Development of Soil Profiling Methods for Forensic Geographical Provenancing

*Catherine Scott, MChem, and Hilary D. Bathgate, BSc, Centre for Forensic Provenancing, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, Norfolk NR4 7TJ, UNITED KINGDOM; Julian Andrews, PhD, and Andrew Lovett, PhD, School of Environmental Sciences, University of East Anglia, Norwich, NR4 7TJ, UNITED KINGDOM; Jonathon Clarke, PhD, John Innes Genome Laboratory, Norwich, NR4 7UH, UNITED KINGDOM; and Jurian A. Hoogewerff, PhD\*, University of East Anglia, Norwich, Norfolk NR4 7TJ, UNITED KINGDOM*

After attending this presentation, attendees will gain an understanding of the different techniques used in the chemical and biological analyses of soil, such as elemental and mineralogical profiling, plant DNA and pollen analysis. Attendees will also be able to observe the results of geographic information system (GIS) modelling for multiple variables across a region.

This presentation will impact the forensic community by introducing a novel method involving multiple parameters for soil analysis to allow the creation of a layered geographic information system (GIS) in order to predict the origin of soil samples.

Locard's principle that "every contact leaves a trace" suggests that everywhere we travel will be recorded by the geological materials retained on our shoes. In this way, the movements of a criminal or an instrument used to commit a crime can also be traced through the analysis of any soil residues found upon on them.

Geological samples, including soils, are frequently analysed by forensic laboratories, usually on a case by case basis, by comparing a suspect sample to an especially collected control. Unlike other materials that can be analysed and compared to a central database, soils as yet cannot be 'matched' to an area unless the area in question has already been identified. There is a need for a method of soil profiling that would allow an unknown sample to be tested and assigned a quantitative likelihood that it originated from a given region. Spatial models can then be created to house data relating to multiple variables and be used to map soils across geographical areas.

Generally, the more variables available with which to compare any two items, the greater the certainty a forensic analyst can have when asserting their similarity and the same applies to geological materials. This poster aims to highlight ongoing research at the Centre for Forensic Provenancing, England, involving the analysis of soil samples collected from across the county of Norfolk. A number of chemical and biological profiling methods will be used to build up a unique signature for soils from different locations. Elemental profiling by x-ray fluorescence spectroscopy (XRF) and inductively coupled plasma-mass spectrometry (ICP-MS) will elucidate any variations in major and trace element concentrations, while isotope ratio mass spectrometry (IRMS) and multi-collector inductively coupled plasma-mass spectrometry (MC-ICP-MS) analyses will determine the light and heavy isotopic signatures of the soils. Mineralogical examination of the samples will be undertaken using x-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Variations in the chemical composition of soils often correspond to changes in the underlying bedrock and soil parent material and these relationships could allow the prediction of values for soils, on the basis of their geographic location.

Biological components of the soil such as plant DNA and pollen can also be used to profile the soil. Although non-human DNA is not yet routinely used, it has helped to link suspects to crime scenes and aided in criminal investigations. Plant DNA from many different species both old and new to the area is often found within the soil; this can be extracted, amplified and then fingerprinted using various techniques such as microsatellites or terminal restriction fragment length polymorphism (TRFLP), to give a unique profile. Palynology, the study of pollen and spores, can also be used in establishing a link between crime scenes and suspects due to the uniqueness of pollen assemblages within a certain area. Pollen is a useful tool because the grains are extremely resistant (the outer walls of pollen grains are composed of sporopollenin, one of the most durable biological substances), and can be found in deposits in which other types of fossils have been diagenically destroyed. As well as pollen assemblages being exclusive to a specific area, pollen grains are produced in enormous numbers and can also be retrieved in great quantities making them an extremely important tool in soil profiling.

Once collected the data will be collated and modelled using geographical information system (GIS) software to map the area under analysis. This technique allows the visualisation of geographical relationships and the combination of different layers of data to determine the most effective parameters for the discrimination of soils. The use of

multiple layers within the GIS should allow a greater degree of certainty when identifying the origin of the soil samples.

**Soil, Profiling, GIS**

## A107  Discrimination of Architectural Paints via Physical and Chemical Methods of Analysis

*Diana M. Wright, PhD\*, Andria H. Mehltretter, MSFS, and Maureen J. Bradley, PhD 2501 Investigation Parkway, Chemistry Unit, Room 4220, Quantico, VA 22135*

After attending this presentation, attendees will be aware of an ongoing FBI Laboratory project to assess the discriminating power of physical and chemical comparisons of architectural paints.

This presentation will impact the forensic community by providing a better understanding of the discriminating power of macroscopic, microscopic, and chemical analyses of architectural paints within the same general color classification.

Architectural paint samples are often submitted to crime laboratories as evidence associated with bank robberies, break-ins, or other violent crimes against persons.  The FBI Laboratory performs comparative physical and chemical examinations to explore the possibility of an evidentiary link between a suspect's personal effects (e.g., clothing or tools) and one or more crime scene(s).

Submitted samples are first evaluated by visual and microscopic means to document physical characteristics.  These include the surface layer's finish (glossy, matte, or eggshell), as well as the number, color, and relative thicknesses of each layer in the evidentiary specimens.  Chemical analysis involves the use of Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDS), and pyrolysis gas chromatography with mass spectral detection (pyGCMS).

This study involves the analysis and comparison of over 900 architectural paint samples utilizing the current FBI Laboratory protocol.  Samples were collected and submitted by Laboratory and FBI field office personnel, as well as forensic scientists from a variety of law enforcement agencies within North America.  Collection sites included interior and exterior surfaces at private homes, commercial buildings, and outdoor public dwellings such as park benches and tables.  Therefore, the sample set represents architectural paints that could be easily accessed by average consumers and would be comparable to evidence commonly submitted to a forensic laboratory for analysis.

Samples were first separated into broad color categories (blue, red, green, brown/beige, yellow/peach, gray/black, and white) through visual and microscopic observations.  Once all samples had been evaluated in this manner, an analyst conducted a pairwise comparison of each sample to all other samples within the same color designation.  Samples that could not be discriminated were carried forward for chemical analysis and comparison.

The objectives of this project include: the determination of the range of physical and chemical characteristics associated with architectural paints, and assessment of the ability of the FBI Laboratory's overall analytical scheme to distinguish between samples.  The analysis and discriminating power of the physical and chemical examination of non-white architectural paints will be the subject of this poster.  Comparable assessments of white architectural paints will be the subject of future work.

**Trace Evidence, Architectural Paint, Discrimination**

## A108  Raman Spectroscopy:  A Solution for the Analysis of Light Colored Fibers?

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After attending this presentation, attendees will understand the principles of raman spectroscopy, the challenges presented when analyzing light colored fibers, the utility of raman spectroscopy for forensic analysis of light blue cotton fibers, and the advantages of raman spectroscopy over current analysis methods for of light colored fibers, specifically microspectrophotometry.

This presentation will impact the forensic science community by proposing a more discriminative method for forensic analysis of light blue cotton fibers and potentially other light colored fiber types, which could result in the successful analysis of fiber evidence that while frequently encountered, remains problematic for current analysis techniques.

Light colored fibers are commonly encountered in forensic case work, but have little probative value due to the difficulties encountered when analyzing them with current fiber color analysis methods, such as microspectrophotometry or thin layer chromatography.  Raman spectroscopy may present an alternative technique for the forensic analysis of light colored fibers.

Raman analysis provides spectral information of fiber colorants based on the inelastic electron scattering that results from the molecular vibrations and rotations when the sample surface is exposed to a laser.  It is a non-destructive technique that requires little sample preparation and can be performed in-situ, all of which are advantages when working with forensic evidence samples.  Previous research has successfully applied raman spectroscopy to the analysis of colorants in fibers and to differentiate fibers within color blocks, however research has been limited to dark colored fibers.  In this research, raman spectroscopy was applied to the analysis of light blue cotton fibers.  Light blue cotton fibers were chosen because blue fibers and cotton fibers are commonly encountered in forensic casework. 22 samples were collected from a variety of clothing types and brands, with only the fiber type known.  The samples were analyzed using Raman spectroscopy and microspectrophotometry.  Two lasers (514 nm and 785 nm) were used for Raman analysis to determine if two different wavelengths elicited additional spectral information.  The samples were classified into groups based on the spectra obtained for each method and the more discriminative method was determined based on a comparison of the resulting groups.  A blind test using four fibers selected from the sample group was also performed.

Raman analysis of the samples resulted in the individualization of 14 out of 22 samples, with three small groups of fibers remaining undifferentiated.  Combining the microspectrophotometry results with those obtained with raman spectroscopy individualized two additional fibers, but microspectrophotometry alone resulted in fewer groups and no individual fibers could be isolated.  The results of the blind test confirmed the utility of raman spectroscopy, which identified two of the unknowns, while none were identified with microspectrophotometry.

Based upon the results obtained, raman spectroscopy was more discriminative than microspectrophotometry for the analysis of light blue cotton fibers.  While analysis with only one laser was more selective than microspectrophotometry, the use of two lasers permitted a higher degree of fiber discrimination.  Overall, raman spectroscopy presents a suitable

alternative to microspectrophotometry that conforms to the needs and demands of forensic science and that could be easily incorporated into the current forensic fiber analysis techniques when analyzing light blue cotton fibers. With further experimentation it could also be applied to light colored fibers of other types. As raman spectroscopy was more discriminative for light blue cotton fibers, it would be most beneficial as a replacement or precursor to microspectrophotometry.

**Raman Spectroscopy, Cotton Fiber, Light Colored**

## A109    Comparative Analysis of Condom Residues Pre- and Post- Coitus by Liquid Chromatography - Mass Spectrometry (LC - MS)

*Lesley Ann M. Huggins*, Peter J. Diaczuk, BS*, and Gloria Proni, PhD*, John Jay College of Criminal Justice, 445 West 59th Street, Science Department, New York, NY, 10019*

After attending this presentation, attendees will know the chemicals present in a pre- and post- coitus vaginal swab in the presence of condoms of different kinds, and lubricants and polymers associated with the presence of different condoms and will have a clear picture of LC - MS in forensic analysis.

This presentation will impact the forensic science community by providing research data of components of different brand condoms.

In the last several years the number of sexual assaults in which the perpetrator used a condom has dramatically increased. In sexual assault cases, lubricants and polymer recovered from the crime scene may provide useful information for the investigation, particularly when DNA evidences are not available. Individuals, generally, use condoms to be protected by sexually transmitted diseases and to prevent identification from the deposited semen. Several techniques have been used in the past to analyze traces left by condoms: raman spectroscopy[1], gas chromatography–mass spectrometry,[2] infrared spectroscopy[3], nuclear magnetic resonance[4], and capillary electrophoresis[5]. In this research liquid chromatography– mass spectrometry (LC-MS) have been used to determine differences between commercially available condoms. The study is organized in two parts. Initially, condoms sold in the United States were solvent - extracted and analyzed by liquid chromatography– mass spectrometry (LC-MS) in order to obtain pre-coital data, which may help to differentiate the condoms. Some of the condoms' brand did contain a spermicide in the lubricant formulation. In the second part of the study the traces obtained from a vaginal swab in post-coital conditions were also analyzed by means of the same technique: the same condom brands have been use in the two parts of the study. Volunteers have been recruited in order to obtain the vaginal swabs after intercourse. The overall goal of the project was to individualize the condoms and consequently collect useful information that could be used in sexual assault cases.

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**Condom Lubricants and Polymers, LC-MS, Vaginal Swabs**

## A110    Discrimination of Candle Wax Materials by Gas Chromatography (GC) and Isotope Ratio Mass Spectrometry (IRMS)

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After attending this presentation, attendees will appreciate the potential of the combination of GC, IRMS and visual techniques for candle wax investigations.

This presentation will impact the forensic community by assisting in objectively evaluating evidence provided by comparison of candle wax materials. This will assist law and court officers in correctly weighing this evidence type in arson criminal investigations and ensuing court procedures.

Candles or candle wax materials are sometimes encountered in arson investigations where they are used in devices to initiate a fire. In this situation often a request is made to compare these candle wax materials with visually similar candles retrieved during *e.g.* a search at a suspect's house. In the past, this was limited to infrared spectrometric (IR) and gas chromatography (GC) characterisation of both wax materials to be compared. If materials could not be discriminated, this was stated but no conclusion could be provided on a common source since the background variation of these characteristics was unknown to us.

In a specific Dutch high profile serial arson investigation it was deemed necessary to both use more techniques as well as obtain a measure of the background variation of the characteristics used. Techniques used at first were visual investigation (color, structure, morphology, layer thickness, hardness), X-ray fluorescence (XRF) to assess low levels of heavier elements, GC to determine aliphatic hydrocarbon profiles and fatty acid levels, GC-MS to identify additional (low-level) compounds that could be used for characterisation and Isotope Ratio Mass Spectrometry (IRMS) to determine isotope ratios of the light hydrogen (H) and carbon (C) elements of the wax samples. In this first part of the investigation it was found that the combination of visual techniques, GC and IRMS was the most informative, apparently discriminating best.

The focus in this investigation was on white or ivory colored common candles without further outside decorations such as embossing. Both tapered as well as cylindrical candles were relevant. These are sold under designations such as household, table, dinner or gothic candles. Depending on the manufacturing process as well as market preferences, candles in general consist of a white or light colored body of candle wax around the central wick and a thin outer layer that may be white or otherwise colored. One of the advantages of this combination is that a manufacturer can use a single type of candle body and cater to market demands for variation (both directly from consumers as well as from

chain stores ordering batches of private label candles) by applying a variation of colored outer layers. Larger producers may in this way produce thousands of colors for their candles. This outside layer will in general have a different composition and a higher melting point than the main candle wax body. When the candle is lit, the outside layer will then melt later than the main body and act as a container for the molten wax material layer from the main body.

The differences in material composition between the main body and the outside layer in principle offer additional characteristics for discrimination. The main wax body typically will be a mixture of paraffins and saturated fatty acids, mostly stearic and palmitic acid. The outside layer almost exclusively consists of paraffins only.

In the second part of the investigation, 130 different boxes of candles were bought in a variety of consumer shops throughout the Netherlands during a short period of three weeks in February/March 2008. The main brands encountered are two different brands from a single Dutch company as well as a number of private label brands from Dutch chain stores. In addition to the direct acquisition of candle materials from the shops, manufacturers and managers at the headquarters of the chain stores were interviewed on potential handles for candle discrimination as well as on buying policies that may influence characteristics variability.

The IRMS and GC analytical methods were validated for these wax materials and candle wax composition variation was determined, both along the length of single candles, within candle boxes and in between candle boxes from a single brand. Apart from the variation in candle wax composition also visual characteristics were determined and it was recognized that candles had been produced using a number of different industrial candle manufacturing processes (extrusion, drawing, molding). The drawing process results in a typical layered structure (tree ring effect) of the candle cross section and in the extruded candle visual inhomogeneities may be observed reflecting the granular nature of the extruder feed.

From the IRMS and GC analytical results it was observed that variation was highest in between brands. For boxes of a single brand, inter box variation was much higher for private label brands than for the main single brand producers. These results reflect buying policies of chain stores for their private label brands. A few manufacturers are selected for a single private label brand to encourage competition and otherwise selected batches of candles may be acquired from other manufacturers.

**IRMS, Candle, Arson**

## A111    An Evaluation on Characteristics of Textile Polymers

*Oho Taick Lee, MSc, Ministry of National Defense, Seoul 140-701 KOREA; Mi-Jung Choi\*, Chungnam National University, 305-764, Daejeon, KOREA; Yale Shik Sun, PhD, Korea Testing & Research Institute, Gimpo, Gyunggi-Do, Gimpo 415-871, KOREA; and Sung-Woo Park, PhD, Chungnam National University, 305-764, Daejeon, KOREA*

After attending this presentation, attendees will have been provided information on the physical characteristic of fiber using microscopic and spectroscopic techniques and chemical characteristic.

This presentation will impact the forensic community by its design to find out how fiber identification is accomplished internally and externally in the particular fiber group.

In criminal investigations, fiber transfer occurs between the suspect, victim and the materials at the crime scene. Synthetic or man-made fibers generally come from synthetic materials such as petrochemicals. But some types of synthetic fibers are manufactured from natural cellulose; including rayon, modal, and the more recently developed Lyocell. Cellulose-based fibers are of two types, regenerated or pure cellulose such as from the cupro-ammonium process and modified or derivitized cellulose such as the cellulose acetates. Fibers are useful traces in the reconstruction of criminal events. The most useful main characteristics are the color, type, diameter, and thickness of the samples, additive material, infrared absorption index as minimally destructive methods and reflected index, solubility, chemical composition as destructive methods. The aim of the presented study is to provide information on the physical characteristic of fiber using microscopic and spectroscopic techniques and chemical characteristic. In case, 30 difference types of cloth sample was collected in characteristics groups. Knit structure of the cloth sample was identified for fiber thickness, degree of fiber damage (spoiled), state of colorants processing as morphologic characteristic, infrared absorption spectra as spectrophotometary of 30 cloth samples, and divided into 10 groups. Also identified were major trace elementary components using ICP. This research is designed to find out how fiber identification is accomplished internally and externally in the particular fiber group through the study on optical microscope fine structure high polymer, micro polymer fiber characteristic by optical microscope, micro polymer fiber characteristic by SEM, micro polymer fiber characteristic by FT-IR, evaluation of fiber element analysis by CCM, analysis of fine element by ICP. This study can supply a minimally destructive(non-destructive) and destructive methology for classification and identification of similarity between samples.

**SEM, Fiber, FT-IR**

## A112    Trace Analysis and Physical Property Characterization of Energetic Materials (Explosives)

*Thomas J. Bruno, PhD\*, National Institute of Standards and Technology, 325 Broadway, Boulder, CO 80305*

The goal of this presentation is to teach an integrated approach to sampling and property measurement for the identification and characterization of explosives in concealed devices. It will also cover the importance of explicitly considering the surface upon which the explosive is deposited.

This presentation will impact the forensic community by demonstrating how the rapid analysis of explosive vapors found in the presence of explosives is crtical to the identification of those explosives, and our new method, called cryoadsorption, provides this. Moreover, we demonstrate that unless the deposit surface is explicitly considered, the results will be incorrect.

Currently, there is a need for standardization, calibration and certification of energetic (explosive) material detection devices. To this end, our laboratory is making quantitative headspace measurements on energetic materials, e.g., trinitrotoluene (TNT), C-4, SEMTEX-A (a plastic explosive made from RDX and PETN (trinitro-triazacyclohexane and pentaerythritol trinitrate, respectively), and detonator cord (lead azide). A headspace measurement is made by a newly developed method called cryoadsorption. The method is implemented by placing a small amount of a material in a sealed vial in a temperature controlled

environment. A capillary is attached to flow He gas into the vial and an activated PLOT column is attached to the vial to allow He gas and other volatile constituents in the headspace to flow out of the vial. The PLOT column is housed mainly in a cryostat that is chilled to ~5°C. The low temperature aids in collecting the constituents of the headspace onto the PLOT column. After a predetermined time the PLOT column is removed, the constituents are collected by flowing acetone through the column, and gas chromatography- mass spectrometry is used to identify and determine the concentration of the analytes. In this presentation, we discuss the identification and concentration of constituents in the headspace of TNT, C-4, Semtex-A and H, Flex-X and detonator cord as a function of temperature. Additionally, we discuss vapor pressure characterization of several common taggents (2-nitrotoluene (2-NT), 3-NT and 4-NT) that have been identified in energetic materials as a function of temperature. Work is also underway to elucidate the permeation of hydrogen peroxide ($H_2O_2$), a major component for making the organic peroxide-based explosive triacetone triperoxide (TATP), through selected polymers.

**Explosive, Analysis, Vapor**

## A113   Chemical Characterization of Solder and Other Metal Components in Improvised Explosive Devices by Laser - Induced Breakdown Spectroscopy

*Erica M. Cahoon, BS\*, Florida International University, University Park, CP 194, 11200 Southwest 8th Street, Miami, Florida 33199; Jose R. Almirall, PhD, Department of Chemistry, FL International University, University Park, Miami, FL 33199*

After attending this presentation, attendees will have learned how chemical characterization by LIBS can provide important information about elemental analysis of metal components and solder used to construct improvised explosive devices.

This presentation will impact the forensic science community by presenting that LIBS be used as a fast screening method, with the potential to be used in the field, for the chemical characterization of trace metal scraps and solder fragments of IEDs.

Pipe bombs and other improvised explosive devices (IEDs) now pose a serious threat to our troops in other countries, as well as to our homeland security. IEDs can be easily assembled, are common weapons of war/terrorism and are being placed in busy public areas, such as, schools, shopping malls, stadiums and other public places. Fast screening methods that can quickly characterize the chemical composition of the components of these devices may assist in the investigation of bombimgs or attempted bombimgs. IED's can be composed of commonly available materials including commercial solder and the other metal components. The IED housing can also become an important item of physical evidence and therefore the chemical analysis of the metal components is an important facet of the forensic investigation. IEDs that have not detonated can be examined to develop investigative leads and to associate the device to a source of manufacture. Trace metal scraps and solder fragments found at the scene can also be gathered post-blast and evaluated for similar reasons. This evaluation needs to be fast and provide the personnel with the chemical characterization that identifies the components of the sample.

Laser-Induced Breakdown Spectroscopy (LIBS) has the potential to assist in the forensic investigation when metal components are required to be characterized. This presentation presents an approach where LIBS can be used as a fast *screening method*, with the potential to be used in the field, for the chemical characterization of trace metal scraps and solder fragments of IEDs. LIBS is a relatively new but emerging method of atomic emission spectroscopy that allows for multi-element and real-time chemical analyses with little or no sample preparation. With the sensitivity of approximately 10-100 mg/Kg (ppm), LIBS is able to chemically characterize metal and solder scraps in-situ. The LIBS instrumentation presented in this work consisted of a 266 nm Nd:YAG New Wave Tempest laser focused onto a sample surface requiring a surface of less than 50 microns in diameter and a single laser pulse therefore making the sampling almost non-destructive. Dual pulse LIBS is constructed in an orthogonal beam geometry where the first pulse was 266 nm ablation followed by 1064 nm Nd:YAG Big Sky laser reheating of the plasma. Chemical characterization methods for metal and solder fragments were developed using peak area and intensity analyses for the proven discriminating element emission lines: Ag, Cu, Zn, Bi, In, Sb, Ce and La, with an Andor intensified CCD detector. Al, Cu, stainless steel and NIST 1131 standards were used in the development of the analytical protocols and to determine the precision, accuracy and repeatability of the LIBS analysis. Elemental profiling, both qualitative and quantitative, will also be conducted using X-Ray Fluorescence (XRF) for comparison and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) for a confirmatory method. Different types of solders and metals were analyzed and results show that LIBS would is a viable method the chemical characterization of solder and other metal components of improvised explosive devices.

**LIBS, Chemical Characterization, Improvised Explosive Devices**

## A114   Analysis of Black Pen Inks by Electrospray Ionization Mass Spectrometry

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This goal of this presentation is to demonstrate a minimally destructive document sampling method with subsequent extraction and electrospray ionization mass spectrometry method for the analysis of dyes and vehicles within pen inks.

This presentation will impact the forensic community by demonstrating how sampling, extraction and analytical methods were established that caused minimal destruction to the document, extracted the ink from the paper, and detected the components within the ink. Ions from the paper did not interfere in the analysis of the ink because they could be substracted from the ink spectra. Identification of the ink components provided information for determination of ink type, i.e., ballpoint, gel, and rollerball. It also provided enough information to distinguish 16 of the 18 inks from one another, however further discrimination might be accomplished by including un - identified ions of the mass spectra.

Paper fibers containing ink samples were removed under a stereo microscope using forceps, thereby causing minimal destruction of the document. Only a few fibers were required to detect the dye components within the ink by electrospray ionization mass spectrometry. However, to detect the vehicle components, an average of thirty fibers was required since the concentrations of the vehicles within the ink are significantly lower than those of the dyes. Typically, methods of ink analysis utilize thin layer chromatography to detect the dyes and gas chromatography to

detect the vehicles which are more volatile than the dyes. Electrospray ionization mass spectrometry (ESI-MS) detects both dyes and vehicles with only a couple of instrumental variations. The detection of the vehicle components is essential for the analysis of inks from gel or rollerball pens, which typically contain pigments rather than dyes. Pigments are insoluble and therefore not detectable by ESI-MS. Identification of the dyes and vehicles contained within each of ink samples provided enough information to distinguish most of the inks from one another.

Eighteen black ink pens were used in this study; six ballpoint, six gel, and six rollerball. An extraction method suitable for all types of pens was examined by varying the solvent and conditions of the extraction. Instrumental methods were investigated by varying several operating parameters such as mobile phase additive, analysis polarity (positive and negative), and applied fragmentor voltage. Mobile phase additives were required to promote ionization of some of the dye and vehicle molecules for detection in the mass spectrometer. Liquid chromatograph pumps were used to supplied mobile phase solvent; however, no chromatography was performed. The sample was introduced into the mobile phase through a standard injection loop and any necessary additives were added into the flow by a syringe pump at a point after sample introduction and before the ESI interface. Multiple injections were performed during a single analytical run including solvents blanks, a control sample of the paper with no ink, and the sample of ink extracted from the paper. This procedure facilitated quality paper background and baseline subtractions from the ink spectra. The resulting corrected spectra were dominated by ions from components in the ink. Eleven papers were examined to determine if ions from the paper interfered with the analysis of the inks. Several dye and vehicle standards were analyzed to identify some of the ink components. Estimated limits of detection of these standards were determined to evaluate the concentration of dyes and vehicles extracted and detected utilizing these methods.

Sampling, extraction and analytical methods were established that caused minimal destruction to the document, extracted the ink from the paper, and detected the components within the ink. Ions from the paper did not interfere in the analysis of the ink because they could be substracted from the ink spectra. Identification of the ink components provided information for determination of ink type, i.e., ballpoint, gel, and rollerball. It also provided enough information to distinguish 16 of the 18 inks from one another, however further discrimination might be accomplished by including un-identified ions of the mass spectra.

**Electrospray Mass Spectrometer, Ink, Document Examination**

## A115 Optimization of a Microwave Assisted Extraction/Headspace Solid - Phase Microextraction (MAE/HS - SPME) Procedure for Organic Impurity Profiling of Seized 3,4-Methylenedioxymethamphetamine (MDMA) Tablets

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After attending this presentation, attendees will be familiar with the application of microwave assisted extraction (MAE) and headspace solid - phase microextraction (HS-SPME) for organic impurity profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets.

This presentation will impact the forensic community by offering an extraction procedure that overcomes limitations in commonly used liquid-liquid extraction procedures and yields more informative organic impurity profiles. With more informative organic impurity profiles, greater confidence is achieved in the association and discrimination of organic impurity profiles from different MDMA exhibits.

Several different methods are used by clandestine laboratories to synthesize MDMA. Because these labs typically do not employ quality control measures, impurities resulting from starting materials and by-products of the reaction are often present in the final MDMA product. Typically, organic impurities are extracted from MDMA using a LLE procedure, and the extract is analyzed by gas chromatography-mass spectrometry (GC-MS), generating an impurity profile of the tablet. Based on the impurities present, the synthetic route used to manufacture the MDMA may be determined since some impurities are route specific. Furthermore, similar levels of the same impurities may imply that the tablets originated from a common clandestine lab. However, the LLE procedure commonly used for impurity extraction requires a relatively large sample mass and often efficiently extracts MDMA which can mask the presence of trace level impurities in the final chromatogram. Due to these limitations, alternative extraction procedures may be more useful to obtain informative organic impurity profiles.

In the presented research, MAE followed by HS-SPME is investigated as a possible alternative to LLE. MAE is first used to extract the organic compounds of the sample into a buffer solution. Because MAE provides highly efficient extractions, all components in the sample that are soluble in the buffer are extracted. The HS-SPME step after the MAE allows for the selective extraction of impurities. Due to the low volatility of the salt form (which is typically present in tablets), MDMA is not efficiently extracted by HS-SPME. In addition, HS-SPME offers the advantage of pre-concentrating impurities on the fiber which is especially important for impurities present at trace levels. Therefore, the resulting organic impurity profile is likely to be more informative.

A factorial experimental design is used to screen for the important parameters in the MAE and to evaluate the interaction of the different parameters. The parameters of the microwave extraction screened in the design include extraction time, extraction temperature, ramp rate, and sample mass. A central composite design is used to optimize the important parameters determined by the screening design. The HS-SPME procedure utilized was previously developed in our lab. The development of the MAE/HS-SPME procedure for the extraction of organic impurities from seized MDMA will be presented and compared to conventional LLE procedures based on the number of impurities extracted and the repeatability and reproducibility of the extraction, as well as the overall chromatography.

**3,4-Methylenedioxymethamphetamine (MDMA),**
**Microwave Extraction, Impurity Profile**

## A116 Detection of Anabolic Steroids and Related Compounds in Black Market Samples

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After attending this presentation, attendees will have been presented with the techniques used by the FDA's Forensic Chemistry Center (FCC) for analyzing black market anabolic steroids and other related compounds.

This presentation will impact the forensic community by presenting an analytical roadmap for those in the general forensic chemistry community who may encounter similar cases.

This presentation will highlight the cases received, as well as the analytical approaches used by the FCC to quickly and accurately identify anabolic steroids, masking agents and related compounds in evidence linked to underground steroid labs.

The Anabolic Steroid Control Acts were passed in 1990 and 2004, placing 59 anabolic steroids, as well as any salt, ester or ether of each, in schedule III of the Controlled Substances Act. Their passage, coupled with America's obsession with body image, has spawned a network of black market steroid labs that gross close to $500 million dollars annually. The FCC receives sample submissions from all over the U.S. in cases related to production, importation, and distribution of anabolics and other pharmaceuticals used in conjunction with these types of steroids. Most recently, the FCC participated in the analysis of some of the 11.4 million steroid dosage units seized internationally as part of Operation Raw Deal, a joint DEA, FDA, and USPIS investigation.

Evidence received by the FCC includes anabolic steroids as injectable preparations, oral dosage forms, and topical creams. In addition, many of these steroids encountered at the FCC are foreign-labeled, mislabeled or unlabeled. The FCC is able to differentiate in excess of 120 different steroids, with testosterone, methandrostenolone, nandrolone, and stanozolol most frequently encountered. Identification of these anabolic steroids and other related compounds is typically performed using different chromatographic techniques coupled with mass selective detection.

Abuse of anabolic steroids by athletes and bodybuilders is often accompanied by the use of other drugs. Commonly encountered with these sample submissions are phosphodiesterase-5 inhibitors (such as sildenafil and its analogs), selective estrogen receptor modulators (such as tamoxifen and clomiphene), and diuretics (such as hydrochlorothiazide and spironolactone). The presence of these other classes of drugs can complicate analyses targeted exclusively at steroids.

These examples are presented as an analytical roadmap for those in the general forensic chemistry community who may encounter similar cases.

**Anabolic Steroids, Black Market, Masking Agents**

## A117 Direct Analysis of Trace Analytes and GSR From Fibers Utilizing Nanomanipulation-Coupled Mass Spectrometry

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After attending this presentation, attendees will learn about a new instrumental technique for trace analysis.

This presentation will impact the forensic science community by allowing analysis of analytes below ppb levels.

A novel instrumentation of nanomanipulation coupled to nanospray mass spectrometry in order to probe trace analytes and gunshot residue from fibers will be presented. Nanomanipulation is ideal for these applications due to its translational resolution of 10-100nm, in lieu of the optical limit, making it ideal to couple to nanospray mass spectrometry, which only requires a minimum of 300nL and 300attograms of analyte. This technique increases analyte detection sensitivity, and decreases the amount of sample required with minimal damage to the evidence. With this instrument we are able to directly probe and manipulate from a fiber using the nanospray tip, and then transfer the analyte to the mass spectrometer reducing the analyte preparation. This technique is demonstrated by probing histidine and caffeine from a single rayon fiber then analyzing the trace particles. Also demonstrated is the extraction of GSR from dyed fabric. The instrument is multifunctional with applications to the forensic sciences including analysis of trace elements, gunshot residue, and document ink.

**Trace Analysis, Mass Sspectroscopy, Nanomanipulation**

## A118 The Scientific Working Group on Dog and Orthogonal Detector Guidelines

*Kenneth G. Furton, PhD\*, International Forensic Research Institute, Florida International University, University Park, Miami, FL 33199*

After attending this presentation, attendees will have a better understanding of how establishing best practices for detection teams will improve interdiction efforts as well as courtroom acceptance of dog alert evidence by improving the consistency and performance of deployed detector dogs.

This presentation will impact the forensic science community by providing a variety of benefits to local law enforcement and homeland security including improved interdiction and courtroom acceptance by improving the consistency and performance of deployed teams and optimizing their combination with emerging electronic detection devices.

The Scientific Working Group on Dog and Orthogonal detector Guidelines (SWGDOG) is a partnership of local, state, federal and international agencies including law enforcement and first responders. This project was undertaken as a response to concerns coming from a variety of sectors including law enforcement and homeland security regarding the need to improve the performance, reliability, and courtroom defensibility of detector dog teams and their optimized combination with electronic detection devices. This project was modeled after the successful precedent of a variety of other scientific working groups (SWG's), SWGDOG being the eleventh since 2005. Presently there are thirteen SWG's as of 2008 all challenged with developing internationally recognized consensus-based best practice guidelines developed by a membership of respected scientists, practitioners, and policy makers representing diverse backgrounds. SWGDOG general meetings have been held biannually for the past four years to produce the initial set of guidelines with NIJ funding the management of this project and the travel for international members. The DHS and FBI have funded the travel and meeting costs for the domestic SWGDOG members for the past four years.

The current success of SWGDOG is being manifest by a shift of some national canine organizations to adopt the approved best practice guidelines proposed. Though SWGDOG guidelines are not mandatory, this positive change is the ultimate goal of the working group. The continued approval and revision of SWGDOG documents has received an increased number of public responses and input which has shaped the documents making them publicly vetted.

The approval of each subcommittee best practice document takes 6 months to complete including a 2 month period of public comments. The

nine SWGDOG subcommittees and target timetable for posting of the best practice guidelines are as follows: (1) Unification of terminology (Part A - April '06; Part B - October '06; Part C - March '07; Part D - August '07; Part E - March '08; Part F – September '08), (2) General guidelines (April '06) - Publication in FSC October '06) First Revision (September '08), (3) Selection of serviceable dogs and replacement systems (October '06), (4) Kenneling, keeping, and health care (October '06), (5) Selection and training of handlers and instructors (October '06), (6) Procedures on presenting evidence in court (October '06), (7) Research and technology (March '07), (8) Substance dogs: Agriculture; Arson; Drugs; Explosives; (August '07) Human remains (September '08), and (9) Scent dogs: Scent identification; Search and Rescue; Trailing dogs; Tracking dogs (Part A - March '07; Part B – August '07; Part C – March '08; Part D – September '08).

Establishing consensus based best practices for the use of detection teams is expected to provide a variety of benefits to local law enforcement and homeland security. Benefits include improved interdiction efforts as well as courtroom acceptance by improving the consistency and performance of deployed teams and optimizing their combination with electronic detection devices. While it is not technically part of the scope of SWGDOG, a future accreditation program based on SWGDOG guidelines will be an important mechanism to facilitate the adoption of these SWGDOG guidelines.

**SWGDOG, Detector Dog, Consensus Guideline**

## A119 Comparison Study of Laser - Induced Breakdown Spectroscopy Using Glass Standards for Trace Elemental Analysis

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After attending this presentation, attendess will become familiar with the applications of LIBS and the conditions for obtaining and the analysis of LIBS spectra.

The impact of this presentation on the forensic community will include corroboration that data collection from one lab can be duplicated by another lab using a similar LIBS configuration (Rodriguez-Celis 08) and an investigation into utilizing trace elements for finer distinction between similar glasses such as automotive glasses.

The objective of this project is to investigate the sensitivity of the LIBS instrument using NIST standards 612 and 610. This is achieved by determining the number of trace elements that can be identified in NIST standards 612 and 610. Once the sensitivity is determined, 31 automobile glass samples will be analyzed for trace elements to determine individuality/uniqueness of these samples. The educational objectives of this presentation are to familiarize the attendee with the applications of LIBS and the conditions for obtaining and the analysis of LIBS spectra.

Modern LIBS instruments use a high power laser pulse to generate a plasma from the sample. Light is given off from the excited species in the plasma as they drop back to their ground state. The light from the different species is collected after an initial delay of approximately 1 μsec. The delay helps reduce the noise detected by the instrument. The collected light is dispersed by a spectrometer or monochromator, and then collected by a detector that sends the data to a computer to be analyzed (Miziolek 06).

Glass analysis was one of the first forensic applications of LIBS to be studied. Currently, density and refractive index are most often used to classify glass for forensic purposes (Saferstein 07). However, as glass manufacture becomes more standardized, the refractive indices and densities can overlap leaving no discernable difference among fragments of glass (Koons 01). Elemental analysis can supplement the analysis of physical properties of glass for greater discrimination among glass types and LIBS provides a quick and non-destructive elemental analysis of glass.

The first objective of this study is to investigate the reproducibility of glass comparisons reported in the literature. In their 2008 report published in Analytical and Bioanalytical Chemistry, Rodriguez-Celis et al. described the methodology for identifying glass from a common automotive source. The analysis consists of 3 important steps:

1. Optimization of the experimental setup to a statistical variation of less than 15%
   a. All data is collected at a constant detector delay of 1 μsec. and integration time of 2.1 msec.
2. Recording 100 spectra each from 15 locations on the glass sample
3. Correlating the linear and rank correlation coefficients to a library spectra (Generated in the lab)

The second objective of this study is to investigate the analysis of trace elements as a way to differentiate between similar types of glasses. As stated previously, the manufacture of glasses is becoming more standardized and there is less variation in glass compositions from manufacturer or even from plant to plant. However, there may be variations in the composition of trace elements from batch to batch within a manufacturer's products. One way to detect trace elements is to vary the detector delay to optimize the signal for certain elements. For example, under standard conditions, the line at 309.3 for Al was very week. When the detector delay was lengthened to 1.5 μsec., the line at 309.3 was enhanced relative to other lines in the spectra.

In conclusion, in this presentation we will report on the ability to apply the experimental conditions from one LIBS system to another lab as well as expanding into identification of trace elements as a means of further discriminating between glasses.

**LIBS, Glass, Elemental Analysis**

## A120 Biomatrica DNA SampleMatrix® – A New Prospect for Forensic DNA Sample Storage

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The goal of this presentation is to address the feasibility of using Biomatrica DNA SampleMatrix® as an effective means of stabilizing extracted DNA for room temperature storage, as well as to discuss the advantages and disadvantages of the product for forensic DNA samples.

This presentation will impact the forensic community by illustrating that there may be a space conserving method for long-term DNA sample storage without refrigeration.

Forensic labs across the country currently store DNA extracts in -20°C freezers for sample preservation in an attempt to maintain their integrity over long tern storage. These freezers take up vast amounts of valuable laboratory space and need to be continuously monitored for

potential malfunctions. Freezing samples may also sheer and damage the DNA, especially with repeated freeze-thaw cycles. As an alternative method of storage, the Palm Beach County Sheriff's Office (PBSO) began researching Biomatrica's DNA SampleMatrix® (SM), which is presently marketed through Qiagen as QIAsafe DNA 96-well plates and individual tubes. In this study, the SM 96-well plates were evaluated against current storage methods at six time points ranging from 1 day to 3 months. Matrix samples were assessed for overall sample recovery and quality versus the in-house controls (IHC). Similarly, SM individual tubes were tested, at three time points ranging from 2 weeks to 4 weeks, and compared to IHC samples. The SM 96-well plate and the SM individual tubes were further evaluated at each time point with respect to two different environmental storage conditions. Samples were stored in identical storage cabinets, one with a humidity controlled environment and the other without humidity control.

Sensitivity and mixture series were utilized for the evaluation of the SM. The sensitivity study consisted of DNA concentrations from 4ng to 0.0625ng in a total of 20µL, with a serial dilution factor of 2.0 for both a male and female donor. The mixture study consisted of 1ng total in 20µL of several different male to female ratios. The Beckman Coulter BioMek® NX$^P$ was used to aliquot 20µL of each sample from a stock tube into SM 96-well plates and SM individual tubes for each time point and condition. The NX$^P$ simultaneously created IHC samples of 20µL aliquots of each sample from the same stock into dolphin tubes. IHC samples were stored in a -20ºC freezer, while the SM samples were dried overnight in a laminar flow hood and stored in their respective conditions.

SM samples were rehydrated with 20µL of autoclaved water for sample recovery. All recovered DNA samples were quantified using Applied Biosystems Quantifiler™ Human DNA Quantification kit on the ABI 7000 and compared to determine if DNA stored on the SM was recovered at the same or higher concentrations than those in the IHC condition. Both the SM samples and the IHC samples were also compared to a baseline created of the original DNA stock tube at the time samples were plated. The remaining sample was amplified using Promega's multiplex STR PowerPlex® 16 system. Once amplified, the samples were run on Applied Biosystems 3130xl Genetic Analyzer to analyze the integrity of the DNA after storage on the SM.

The integrity of the sensitivity samples was determined by observing the complete loci calls and comparing the average relative florescence unit (RFU) values of each allele at each locus. Complete loci calls were determined by overlapping the replicate electrophoregrams for each sample. The mixture samples were evaluated by observing the complete loci calls from each donor, whether a minor contributor could be identified, and if dropout was observed.

The data shows SM individual tubes did not perform as well as the SM 96-well plates. Analysis of the qPCR data is currently being completed to determine if DNA can successfully be stored at ambient temperatures. Further studies need to be conducted to evaluate if DNA can be stabilized at periods longer than 3 months, and what effects of repeated cycles of rehydrating and drying of the SM would have on the DNA sample.

**DNA Storage, DNA SampleMatrix®, Biomatrica**

## A121   Recovering DNA Profiles From Low Quantity and Low Quality Forensic Samples

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After attending this presentation, attendees will learn how the use of reagent PCRboostTM (Biomatrica, San Diego, CA) can optimize methods for recovering DNA profiles from low quality and low quantity forensic samples.

This presentation will impact the forensic community by explaining why millions of biological samples, including cells, viruses, and DNA/RNA, are stored every year for diagnostics, research and forensics. Forensic evidence samples such as hairs, bones, teeth and sexual assault evidence may contain less than 100 pg of DNA. Low DNA yields may be due to damage or degradation, small cell numbers found in low copy number (LCN) or 'touch' samples, oligospermic or aspermic perpetrators, or low male DNA from extended interval postcoital samples in sexual assault cases. Trace biological evidence including fingerprints also provide low yields. Optimal methods for recovering DNA profiles from these types of low quality and low quantity forensic samples is critical to downstream analysis or re-testing.

Successful forensic analysis depends on the ability to identify and individualize biological evidence. Many forensic evidence samples such as hairs, bones, teeth and sexual assault evidence often contain less than 100 pg of DNA.[1,2] Low DNA yields may be due to damage or degradation,[1,2] small cell numbers found in low copy number (LCN) or 'touch' samples,[1-5] oligospermic[6] or aspermic perpetrators,[7] or low male DNA from extended interval post-coital samples in sexual assault cases.[8] Trace biological evidence, including fingerprints, also provide low yields of DNA.[9-11] Degradation is another factor that can contribute to further damage compromised sample types, including those derived from ancient or degraded bones or teeth.[12,13] Degradation results in reduction or loss of the structural integrity of cells and proteins, which in turn affects the quantity and quality of recovered nuclear and mitochondrial DNA (mtDNA). Sub-optimal storage can also detrimentally affect sample integrity. Reduction in DNA recovery has been observed with refrigerated liquid DNA extracts and also those exposed to multiple freeze-thaw cycles; the loss may be exacerbated by the use of certain microfuge tubes.[14,15] Therefore, the development of optimal methods is critical for successful recovery of DNA profiles from these types of low quality and quantity forensic samples, particularly if downstream analysis or re-testing is necessary.

Low yields or loss of DNA due to these and other factors may preclude or diminish the ability to test LCN crime scene samples using current STR methods, thus mtDNA testing is typically dictated for low quantity samples suffering from advanced states of degradation. Forensic PCR protocols typically specify 1.0 ng of DNA for optimal

amplification.[16] However, the quantity and quality of template DNA from typical low copy forensic samples falls below this requirement. Furthermore, samples may also contain inhibitors to PCR that co-extract with the DNA, resulting in sub-optimal amplification reactions providing partial profiles or no typing, thereby greatly reducing the probative value of the samples. Modifications to existing amplification and typing protocols (e.g., mini amplicons, whole genome amplification and LCN protocols) to increase the DNA signal are currently being investigated to increase the analytical success rate of challenged samples.[17-19] However, complete forensic DNA profiles are not always achieved when the samples are extremely low quantity and quality.

A method was recently reported where inclusion of a novel reagent, PCRboost™ (Biomatrica, Inc.) was able to enhance amplification 5-fold or more of challenging and difficult to amplify samples. This study aims to evaluate the use of PCRboost for forensic DNA analysis to enhance amplification and recovery of forensic DNA profiles from low quantity and low quality samples.

This study will be conducted in three phases: (1) Amplification of control DNA (including 9947a) using serial dilutions down to pg amounts; various formulations of PCRboost will also be evaluated, (2) Amplification of damaged, degraded and low copy DNA samples including non- probative bone and teeth samples, and (3) Amplification of DNA containing varying amounts of inhibitors. Other experiments including amplifcation of mixtures will also be performed. Results from preliminary experiments conducted by members of an inter-laboratory consortium will be presented.

Amplicons from multiplex STR and/or mtDNA amplification will be assessed by capillary electrophoreisis using the Applied Biosystems 310 or 3130 genetic analyzer. Analysis of the data will be performed using the new GeneMapper ID software from Applied Biosystems. Assessment of qualitative and quantitative data of all samples will be evaluated using GMID software. Analyses of the replicates of each set of data will be performed and statistical analysis will be done to rigorously evaluate and assess any differences between control and test samples with and without PCRboost.

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**DNA, PCRBoost, Forensic Samples**

## A122    Use of a Novel Enzyme to Prepare Samples for Forensic Analysis

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The goal of this presentation is to demonstrate the use of a new enzyme for quick and easy preparation of DNA from various types of forensic samples.

This presentation will impact the forensic community by presenting data on the use of a novel enzyme for the treatment of various types of forensic samples for DNA analysis by STR typing. The procedure in general involves a brief digestion with an endopeptidase isolated from a novel extremophile organism from the Antarctic. The process can be carried out in a single tube with no transfers involved, thus minimizing chances for contamination or sample switches.

An expanding area in forensic DNA profiling involves databases of reference DNA profiles from known individuals. These profiles can be compared against others from crimes with no known suspect. Correspondence between a sample and the database allows linkage of an individual to a crime-scene and this may lead to a conviction. Reference DNA databases are usually compiled from past offenders or suspects (depending on jurisdictional legislation) and most often use buccal swabs to obtain reference profiles. There is therefore a need for a simple method that is easy to automate to help address the backlog of samples waiting for CODIS database testing.

For most DNA forensic analyses, the quality of DNA extracted directly affects the ability to obtain high quality forensic DNA profiles. The DNA extraction procedures commonly used in the forensic field tend to be time-consuming, costly, involve potentially toxic chemicals, and may involve repeated sample transfers which expose the sample to potential contamination – a feature that often leads to trepidation in forensic biology. Alternative strategies have been devised by many manufacturers (for example spin columns or magnetic beads), but these tend to be more expensive and still involve multiple transfer steps.

Data will be presented involving a novel DNA extraction method which can be carried out directly on forensic samples in a single tube with little or no sample transfer. The method is also easily automatable. The premise of these methods is the use of a broad specificity endopeptidase that can be added to forensic sample preparations and which remains minimally active until the sample reaches a temperature of 75°C. Upon reaching its activation temperature, the enzyme digests all proteins, including nucleases, that would interfere with downstream analysis. After this incubation, the preparation is brought to 95°C, whereupon the endopeptidase is totally and irreversibly inactivated. The resulting DNA solution can then be amplified by standard methods to obtain STR profiles.

This novel endopeptidase can be incorporated into kit form for sample preparation. Data obtained from such preparations will show results of DNA extraction from crime samples with optimized methods and formulations providing PCR-ready DNA in just over 30 minutes. Such preparations offer a gentle method which prevents release of inhibitors into the extracted DNA solution while maintaining a closed-tube state. This process works to minimise the risk of extraneous contamination by foreign DNA. Kits may be specifically designed to be compatible with common STR profiling kits and forensic genotyping methods.

Comparisons of the novel endopeptidase methods with the currently validated methods used for forensic samples were carried out. Sample types tested included buccal swabs, blood (whole and stain), and others, including low DNA copy number samples. Comparison of these isolation methods included several commercially available DNA quantitation kits and STR kits. The results of these comparisons will be presented.

**Sample Preparation, STR Analysis, Novel Method**

## A123    Validation of the Quantifiler® Duo DNA Quantification Kit

*Tracey E. Wright, BS\*, Virginia Commonwealth University, 1000 West Franklin Street, Richmond, VA 23284*

After attending this presentation, attendees will acquire information about the Quantifiler® Duo DNA Quantificiation Kit from Applied Biosystems.

This presentation will impact the forensic community by assisting other labs in deciding if integrating the Quantifiler® Duo DNA Quantification kit will enable the laboratory to quantify the amount of total DNA and male DNA in a sample.

This presentation will provide more information or understanding about the Quantifiler® Duo DNA Quantificiation Kit from Applied Biosystems. This kit utilizes the enhanced multiplexing technology of the Applied Biosystems 7500 Real-Time PCR System and the Sequence Detection Software v.1.2.3 in order to simultaneously detect human and male DNA in samples that are commonly encountered in forensic laboratories. To better understand both the attributes and limits of the Quantifiler® Duo DNA Quantificiation Kit, a series of validation experiments were carried out to test precision, reproducibility, sensitivity and mixture interpretation, for its possible casework implementation at the Westchester County Forensic Laboratory.

The precision of the Quantifiler® Duo DNA Quantificiation Kit was determined through examining the cycle threshold number for the dilutions of the DNA standard provided with the kit. The quantitation values reported for the dilutions of the National Institute of Standards and Technology (NIST) standards were used to establish the kit's ability to provide reproducible results intra- and inter-plate. The NIST male quantitation standard was serially diluted to approximately 1.44pg/μL to test the kit's sensitivity. Mixtures were prepared from known male and female samples. Ratios of male to female DNA were made to assess the detection capabilities of the male component relative to increasing amounts of female component. Additionally, fourteen mock casework samples were extracted using a differential extraction procedure and quantitated using the Quantifiler® Duo kit.

Further investigation was done to see if results obtained using the Quantifiler® Duo DNA Quantificiation Kit had any direct effect on the subsequent DNA profiles detected. This was done by amplifying the sensitivity, mixture, and mock samples with the AmpfℓSTR® PCR Amplification Kits and subsequently placing them on the Applied Biosystems 3130 Genetic Analyzer and analyzing them using GeneMapper v3.2.

Quantifiler® Duo DNA Quantificiation Kit was found to be both precise and reproducible. The kit was sensitive to DNA concentrations of approximately 11.5pg/μL. It detected the male component down to a male to female mixture of 1:200. The kit functioned well with the mock

casework intimate samples that were differentially extracted. The reported DNA quantities of the sperm fraction were consistent in terms of both the total human DNA and the total male detected (total DNA ~ male DNA). In many of the epithelial fractions, both male and female contributors were indicated.

Overall, the Quantifiler® Duo DNA Quantification Kit enables the laboratory to quantify the amount of total DNA and male DNA in a sample. Furthermore, results obtained can be useful in enhancing the profile of a male contributor in a mixed sample when it previously could not be detected using the current standard human DNA quantitation method. However, it should be noted that there are some limits to the kit's sensitivity regarding the upper and lower limits of detection. The results obtained from this internal validation will hopefully help other labs in the forensic community decide if integrating the Quantifiler® Duo DNA Quantification Kit enables the laboratory to quantify the amount of total DNA and male DNA in a sample. Furthermore, results obtained in their laboratory would be beneficial.

**Quantifiler Duo, DNA Quantification, 7500 Real-Time PCR System**

## A124 Atmosphere Pressure Glowing Discharge Ionization Source - Ion Mobility Spectrometry for OnSite Analysis of Perfume Odors

*Lei Heng\*, Liaoning Police College, 199 Yingping Road, Ganjingzi District, Dalian, 116023, PEOPLES REPUBLIC OF CHINA*

This presentation will impact the forensic science community by application of on - site analysis of Ion Mobility Spectrometry.

The goal of this presentation is to introduce an atmosphere pressure direct current glowing discharge ion source coupled to an ion mobility spectrometer for on-site analysis of volatile organic compounds (VOCs) directly. This presentation will impact the forensic community by demonstrating a novel ion ionization source when coupled to ion mobility spectrometer will provide forensic examiners a new tool to analysis VOCs compounds which can be hardly detected by traditionally 63Ni ion source, which is quite useful for on-site analysis of perfume odors and to search for hidden flammable liquids.

Ion mobility spectrometry was developed as a technique that utilized the differences of ion mobility in electric field to separate and determine chemical substance in gas phase. The theory of ion mobility is to characterize different chemical materials based upon the mobilities of gas phase ions in a weak electric field and has been successfully used in the detection of latent traces of explosives, illicit drugs, pesticides and chemical warfare agents. In this presentation, the characteristic of a new ion mobility spectrometer is demonstrated that this is a new way for on – site analysis of odor substances

A home-made ion mobility spectrometer with direct-current glow discharge ionization source (DCGDS) was used to analyze perfume odors without any collection and concentration process, the spectrometer was built by Dalian Institute of Chemical Physics, Chinese Academy of Sciences. The analysis was performed in positive mode with following experimental conditions The drift gas flow was kept at 550 mlmin$^{-1}$ and carrier gas at 200 mlmin$^{-1}$, both of which were purified air. The electric field strength was 210 Vcm$^{-1}$. The temperature of the drift tube was 298 K, the atmospheric pressure was 101.3 Kpa, and the lenth of drift tube was 110 mm.

The advantages of IMS technique for gaseous material analysis are simple and convenience, so the sample inlet part of the method is very import. There are many inlet methods, like syringe sampling, or inhale the gas sample directly to the IMS instrument with a pump, a small sampling pump was used in this study.

The types of perfume can be identified by reduced mobility value $K_0$ and peak characteristics, which are obtained from the positions of ion fragment peaks and intensity; and the characteristics of different types of perfume can also be identified.

In summary, the ion mobility spectrometry with glow discharge ionization source is an effective technique in the perfume odor detection on site or in laboratory, it also will be a new way of gas sample analysis and a new solution to collect and analyze the odor material evidence on scene.

**Ion Mobility Spectrometry, Odors, Identification**

## A125 Optimization of Explosives Analysis by Gas Chromatography and Liquid Chromatography

*Jack Cochran, BS, Kristi Sellers, BS\*, Rebecca Wittrig, PhD, and Michelle Misslewitz, BS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823*

After attending this presentation, attendees will understand how to shorten analysis time and employ GC-MS (gas chromatography-mass spectrometry) using a vacuum - outlet GC column configuration for analyzing explosives. Attendees will also understand how to apply HPLC (high performance liquid chromatography) using a dual column confirmation method for analyzing the same explosives.

The instrument set up and analysis methodology discussed will impact the forensic community by providing two means of processing and analyzing explosives using chromatography.

The analyses of explosives have become increasingly important due to the current threat of both home-made and military-grade explosive devices. Nitroaromatics, nitramines, and nitrate esters are used as explosives, are byproducts of the manufacturing process, and can be degradation products transformed by the explosion. These compounds are of environmental concern and, thus, forensic concern due to their carcinogenic, mutagenic and toxic effects. Traditionally, the expired munitions have been disposed of by combustion, resulting in a significant amount of contamination in soil and groundwater. Two United States Environmental Protection Agency (EPA) methods (EPA 8095, 8330b) have been validated to analyze these toxic compounds and can be used as a guide for forensic analysis.

EPA method 8095 focuses on explosives analysis by gas chromatography (GC). This methodology highlights the use of the electron capture detector (ECD). This type of detector is useful due to its selectivity and low limits of detection for halogenated compounds and for compounds with electronegativity characteristics. However, another powerful detector that can be utilized for this type of analysis is mass spectrometry (MS). In this case, vacuum-outlet GC can be applied to EPA method 8095 using a MS detector. This column configuration solves two of the most prominent problems when analyzing explosives. The first challenge is to move the compounds through the column quickly. This can be alleviated by using a short (6m), wide bore (0.53 mm) analytical column. Another problem that quickly arises is the proper flow conditions (1-4 mL/min) needed for the mass spectrometer. By attaching

a short (50-100 cm) narrow bore (0.1 mm), guard column, flow rate can be reduced significantly eliminating this problem. The vacuum-outlet GC column configuration reduces the analysis time from approximately 25 minutes to 3 minutes. In addition, the mass spectra produced by this analysis can undisputably identify the explosives of interest.

High performance liquid chromatography (HPLC) analysis of explosives is highlighted in EPA method 8330b. This methodology HPLC employs analysis with a dual wavelength (210 and 254 nm) UV detector and using a dual column set up. This dual column confirmation analysis is typically done on a C18- type column as the primary column and with a cyano or phenyl-based stationary phase as the confirmation column. By using two columns with different stationary phase selectivity, analysts can more accurately identify or confirm the compounds of interest. In this study, various stationary phases were evaluated for retention and selectivity of all method analytes, and a column pair was identified for this analysis.

In conclusion, the methods developed in this study can benefit analysts by providing two fast and reliable chromatographic methods for the analysis of explosives, GC-MS and HPLC.

**Explosives, GC-MS, HPLC**


## A126 Evelution and Validation of Purification Columns for Forensic DNA

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The goal of this presentation is to evaluate four purification columns for forensic DNA analysis.

This presentation will impact the forensic science community by allowing them to evaluate multiple DNA purification columns for the replacement of the Millipore Centricon columns.

A crucial aspect in the field of forensic science is the ability to concentrate and purify extracted DNA. Millipore Centricon concentrators are popular due to their efficiency and ease of use. Unfortunately they are no longer being produced so an alternative must be evaluated.

In this experiment four columns were evaluated: Millipore Microcon, Sartorius-Stedim Vivacon-2, Pall Microsep and Millipore Microcon. The columns were evaluated based on the quality and quantity of DNA collected and ease of use. It is hypothesized that one of the concentrators will perform equally or better than the Centricon columns. A variety of biological samples were chosen to represent those encountered in forensic casework. Samples were extracted using a phase separation extraction method and concentrated with the columns in parallel. Samples were then quantified using real-time quantitative PCR (Quantifiler, ABI 7500, Applied Biosystems) and amplified using Identifiler (Applied Biosystems) STR multiplex. The amplicons were detected using a genetic analyzer (ABI 310).

This experiment determined that the overall performance of the four columns tested were all good and very similar. Based on ease of use and performance the Vivacon-2 was the most easily manipulated. The Vivacon-2 column was found to retain the highest amount of DNA from the majority of samples tested. Further experiments will be conducted to test the reproducibility of results for each column. Results of the evaluations of all four columns will be presented. Funding for this project was provided by NSF-REU.

**DNA Purification, Concentrator Column, DNA Extraction**


## A127 A Comparison of Two Real - Time PCR Systems for the Simultaneous Quantitation of Total Genomic DNA and Human Male DNA

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After attending this presentation, attendees will have a better understanding of the abilities of two different commercial real-time PCR dual quantitation kits, Promega Plexor HY, and Applied Biosystems Quantifiler Duo, to simultaneously quantitate total genomic DNA and human male DNA, overcome inhibition, and determine mixture ratios.

This presentation will impact the forensic community by comparing two products of equal purpose in an effort to assist laboratories in their determination of which one will most likely fit their current system.

DNA quantitation is an important aspect of forensic sample evaluation as it determines the amount of DNA necessary for successful STR amplification and analysis. Duplex real-time qPCR systems are an improvement to singleplex quantitative assays, which only quantitate either total human or total male DNA, as they simultaneously quantitate total genomic DNA and human male DNA in a single reaction. A large advantage to dual quantitation is that is allows for less consumption of sample, which is especially important in cases where only a limited amount is available. Additionally, duplex qPCR assays assess the presence of inhibitors and determine the relative female to male DNA ratios of mixed samples.

Several sample types were used to draw a comparison between Promega Plexor HY and Applied Biosystems Quantifiler Duo. The systems were used to quantitate samples of known DNA concentrations, ranging from 200-0.012 ng/mL. Reported human and male DNA concentrations were more concordant with Quantifiler Duo than with Plexor HY, especially for concentrations between 50-0.023 ng/mL. In a study using samples inhibited by denim, dirt, and leather, Promega Plexor HY showed a greater ability to overcome inhibition, as it reported DNA quantities greater than both Quantifiler Duo and Quantifiler Human. A mixture study of female to male DNA at varying ratios resulted in Quantifiler Duo reporting human and male DNA quantities more concordant with the expected values than Plexor HY. Additional results were obtained from known and non-probative samples meant to simulate actual forensic casework specimens. These assays both look to address the shortcomings of singleplex qPCR systems, but differ in their ability to accurately quantitate DNA, overcome inhibition, and report female to male ratios in mixtures.

**Quantitation, Genomic DNA, Male DNA**

## A128   Recovery of Mitochondrial DNA From the Attached Side of Self-Adhesive Stamps

*Katherine A. Roberts, PhD, and Donald J. Johnson, MS\*, California State University at Los Angeles, School of Criminalistics, 5151 State University Drive, Los Angeles, CA 90032; and Meiling Cabral, MS, 5510 Monterey Road, Los Angeles, CA 90042*

After attending this presentation, attendees will have a better understanding of the potential to recover mtDNA from self-adhesive stamps

This presentation will impact the forensic community by demonstrating that it is possible to obtain genetic profiles from self-adhesive stamps

The ability to recover a genetic profile from the backs of self-adhesive stamps holds significant implications to the field of forensic science. This knowledge is pertinent in cases in which stamp evidence is commonly encountered; examples include extortion, threats, and kidnapping, where identifying the individual source of DNA may prove pivotal in criminal investigations. In 1994, the U.S Postal Service discontinued the sale of water-activated stamps and replaced them with a pressure sensitive self-adhesive stamp. The self-adhesive stamp provides an alternative evidentiary source of DNA—DNA from fingerprint residues or "touch" DNA. Thus, there is a need to develop a method to successfully obtain a DNA profile from this alternate source of evidence.

In order to determine the feasibility of recovering "touch" DNA from self-adhesive stamps, research subjects were instructed to affix self-adhesive stamps to envelopes and postcards. Prior to extraction, the image-side of the self-adhesive stamps was exposed to UV light for 10 minutes to decontaminate the external surface of the stamp. The stamps were extracted with phenol: chloroform: isoamyl alcohol and the extracts were purified and concentrated using Centricon® 100 microconcentrators. The extracted products were amplified and a haplotype was obtained using the LINEAR ARRAY™ Mitochondrial DNA HVI/HVII Region-Sequence Typing kit.

Four hypotheses were tested to examine the factors that may influence the recovery of mtDNA profiles from the attached side of self-adhesive stamps. The recovery success for each research subject was calculated to determine whether the recovery of mtDNA profiles varies among subjects. In addition, Chi square analysis was performed to test the null hypothesis that there is no difference in recovery between self-adhesive stamps affixed to envelopes in the morning as opposed to the afternoon/evening. Chi square analysis was also used to evaluate the null hypothesis that there is no difference in recovery between freezer-stored and mailed samples. Finally, Chi square analysis was performed to test the null hypothesis that there is no difference in recovery between self-adhesive stamps affixed to envelopes v. postcards

**Self - Adhesive Stamps, mtDNA, Decontamination**

## A129   Real - Time Quantitative PCR Assay for Mitochondrial DNA Quantification

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After attending this presentation, attendees will be informed regarding a sensitive and accurate method for determining mitochondrial DNA (mtDNA) copy number in forensic DNA extracts and for assessing if such extracts contain PCR inhibitors. Attendees will also gain knowledge regarding the minimal number of mtDNA copies required for successful mtDNA amplification and subsequent sequencing.

This presentation will impact the forensic community by potentially increasing the success rate of mtDNA amplification and sequence analysis and by reducing unnecessary consumption of forensic DNA samples so that retesting may be possible if desired.

MtDNA sequence analysis is a useful analytical tool for analyzing limited quantity and/or highly degraded samples, such as hair shafts, bones, and teeth, as well as being informative in maternal lineage cases. There have been efforts to improve the sample preparation portion of mtDNA analysis by attempting to determine the amount of mtDNA contained within a sample. In addition, a better appreciation of the minimum amount of mtDNA required for successful typing would minimize consumption of evidence. Such knowledge would enable an assessment of the likelihood of generating mtDNA profiles from forensic samples. This presentation describes a highly sensitive real-time quantitative PCR (QPCR) assay which was developed to accurately quantify mtDNA for these purposes.

The target sequence for the assay is located within the mtDNA NADH dehydrogenase subunit 5 gene. The chosen sequence possesses minimal sequence homology to the mtDNA of other forensically-relevant species. In addition, the primers and probe utilized in the QPCR hybridize to invariant regions within the human mtDNA genome (based on current population data). The amplicon generated is small in size (105 bp) making the assay more amenable to quantifying samples which contain degraded DNA. The assay is based on absolute quantification and exhibits high sensitivity enabling the detection of as few as 10 mtDNA copies (0.17 fg). Quantification by this method covers a wide dynamic range up to seven orders of magnitude (i.e., 100 million mtDNA copies or 1.7 ng of mtDNA).

To increase the quality and robustness of the assay a novel, synthetic DNA positive control standard was employed in lieu of a plasmid generated standard. The synthetic standard was designed to contain a unique short sequence so if there was contamination due to the control sample it would be readily detectable. Using a synthetic standard instead of a plasmid-generated standard has several benefits, including enhanced quality control, greater purity, lower cost, higher yield, and easier and timelier production. The assay requires only 2µl of sample and results can be obtained within 40 minutes. An internal positive control to detect the presence of PCR inhibitors can be readily incorporated into the assay. Results of validation of the QPCR assay will be presented.

Experimental studies which correlated mtDNA quantities to mtDNA hypervariable (HV) region amplicon yields revealed that as few as 1,000 copies of mtDNA are required for successful downstream HV analysis.

This observation may serve as a guide for minimizing sample usage during HV amplification thus conserving DNA samples where possible.

The QPCR assay described is reliable, robust, and reproducible and will enable the accurate and precise quantification of mtDNA for use in downstream analysis.

**Mitochondrial DNA, QPCR, Synthetic Standard**

## A130   Implications of a Modified Extraction Method for Degraded Human Skeletal Remains

*Suni Edson, MS\*, Odile Loreille, PhD, and Suzanne M. Barritt, MS, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Rockville, MD 20850; Alexander F. Christensen, PhD, Joint POW/MIA Accounting Command, Central Identification Lab, 310 Worchester Avenue, Hickam AFB, HI 96853; and Michael D. Coble, PhD, and Louis N. Finelli, MD, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Rockville, MD 20850*

After attending the presentation, attendees will learn a new method for extracting DNA from skeletal remains. Attendees will learn how the implementation of this method in a working laboratory has reduced the number of samples unable to be reported.

This presentation will impact the forensic community by providing attendees with the information to improve their own extractions of DNA from skeletal remains. An increase in the number of reportable samples will lead to an increase in the number of individuals identified, as skeletonized remains are often unknown persons.

The Armed Forces DNA Identification Laboratory (AFDIL) routinely processes the osseous remains of United States servicemembers and civilians from past military conflicts for generation of mitochondrial (mtDNA) profiles. These remains are submitted by the anthropologists of the Joint POW/MIA Accounting Command – Central Identification Laboratory (JPAC-CIL) to aid in the identification and/or re-association of skeletal elements by comparison to mtDNA profiles generated from reference materials. Despite recovery from variable environments, examination of the results of more than 4,000 individual fragments has shown that certain, more compact elements have a greater rate of success when processed for mtDNA (Edson, et al., 2004 & 2005). Targeting the better skeletal elements from which to gather mtDNA has allowed scientists from both AFDIL and JPAC-CIL to more efficiently identify the remains of missing personnel.

In 2006 a new protocol for extraction was implemented at AFDIL (Loreille, et al., 2007). Prior to this, a scientist used 2.0-2.5g of pulverized bone incubated overnight at 56ºC in a solution containing an extraction buffer (10mM Tris, pH 8.0, 100mM NaCl, 50mM EDTA, pH8.0, 0.5% SDS) and 100ul of 20mg/ml Proteinase K. The new protocol decreases the input of pulverized bone to 0.20-0.25g. Incubation overnight remains the same, but the solution now contains a demineralization buffer (0.5M EDTA, pH 8.0, 1% N-Lauroylsarcosine) and 200ul of 20mg/ml Proteinase K. Both protocols use an organic extraction for purification following the incubation.

After implementation of this new method, the failure rate of samples decreased from 20% to 5%. This presentation will show the original success rates of 5886 skeletal elements processed using extraction buffer and 736 elements extracted using the demineralization technique. While this method decreases the importance of sample selection, it does not remove it. Initial size and quality of the sample should still be considered. However, a wider range of elements may now be routinely selected for processing rather than attempting to limit selection to the best possible one available; thereby potentially increasing the number of individuals identified.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the U.S. Department of Defense.

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**Mitochondrial DNA, Skeletonized Remains, Demineralization**

## A131   Y-STR Typing Strategy for Challenging Samples: Validation and Application to Historical Cases

*Kimberly Sturk, MFS\*, Odile M. Loreille, PhD, Suzanne M. Barritt, MS, Michael D. Coble, PhD, and Jodi A. Irwin, MS, Armed Forces DNA Identification Laboratory, 1413 Research Boulevard, Building 101, 2nd Floor, Rockville, MD 20850*

After attending this presentation, attendees will be familiar with an aggressive amplification protocol to obtain Y-chromosomal data from degraded skeletal remains. Attendees will learn the results of various experiments conducted to validate this protocol, in addition to learning how this method has already been applied to cases at AFDIL.

This presentation will impact the forensic community by describing a genetic assay that can complement or even replace DNA-based methods currently used in the identification of missing persons.

The identification of degraded skeletal remains at the Armed Forces DNA Identification Laboratory (AFDIL) is primarily achieved by mitochondrial DNA (mtDNA) typing. However, the forensic utility of mtDNA data is limited by the molecule's uniparental inheritance and lack of recombination, which can sometimes result in a low power of discrimination. Mitochondrial DNA testing also requires either direct or maternal references for evidentiary comparison, and in some cases these types of references are unavailable. When specific limitations of mtDNA testing such as these are encountered, data from alternative DNA markers in the nuclear genome would benefit the overall identification effort. Unfortunately, the poor quality and limited quantity of nuclear DNA present in degraded skeletal remains has historically restricted the use of autosomal and Y-chromosomal short tandem repeat (STR) data in such cases. Recently, modified or so-called "low copy number" (LCN) STR typing protocols[1] have shown great promise on the degraded skeletal elements typically encountered at AFDIL[2], particularly when the modified amplification is coupled with an improved DNA extraction.[3]

As a result, AFDIL has begun to validate a LCN STR typing strategy for Y-chromosomal loci.

The application of Y-chromosome STR (Y-STR) typing to degraded skeletal samples can provide additional genetic information that may assist in missing persons investigations. For many cases submitted to AFDIL, Y-STR typing will be valuable in confirming gender, reassociating commingled skeletal elements and, of course, supporting identifications. In fact, the option of testing Y-STRs in these cases should facilitate the overall identification effort by expanding the pool of potential family references that can be used for DNA comparisons. Although Y-STRs do not provide the discriminatory power of autosomal STRs, the fact that distant paternal relatives can provide reference material is of great importance in these decades-old cases for which the necessary family references are unavailable for standard autosomal and mitochondrial comparisons. Additionally, data interpretation issues typically encountered with autosomal STRs from poor quality specimens, such as peak imbalance, allele drop-out and allele drop-in at potentially heterozygous loci, tend to be reduced for Y-STRs because of the haploid nature of the Y-marker.

The commercially-available Y-STR amplification kit used at AFDIL includes 17 loci located on the non-recombining portion of the Y-chromosome and targets amplicons ranging in size from 90 to 330 base pairs. For most STR kits, the optimal template input is approximately 1.0ng. However, the degraded skeletal elements typically processed at AFDIL yield too little DNA to produce usable data with standard amplification conditions. As a result, modifications have been made to the manufacturer's suggested amplification protocol. The recommended Taq concentration has been doubled to overcome potential inhibition from the large volumes of extract added to the PCR (extract volumes were maximized in order to maximize allele sampling and recovery). In addition, the standard polymerase chain reaction (PCR) cycles has been increased by six (for a total of 36 cycles) to facilitate maximum allele detection from limited amounts of amplification template. In order to confirm data authenticity, all amplifications are conducted in triplicate and only alleles observed in the majority of amplifications are included in any finalized, consensus profile [1].

As part of the validation process, the modified Y-STR amplification protocol was evaluated for sensitivity, mixture detection and effectiveness on non-probative case samples. Data generated with both the standard and the modified protocols were utilized to characterize the overall authentic data recovery. The results of these experiments and the forensic implications of these results will be presented. Finally, in order to demonstrate the practical utility of the modified Y-STR typing strategy in cases regularly encountered at AFDIL, a number of interesting historical cases that have benefited from Y-STR data will also be presented.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

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**Short Tandem Repeat, Y-Chromosome, Low Copy Number**

## A132  A Y-STR Mixture Frequency Estimator in Forensic Casework

*Lisa A. Gefrides, MS*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will understand the steps needed to create and use an Excel template for generating statistical frequency estimations with Y-STR haplotypes. The audience will be led through actual casework examples of template use and be shown that a simple, yet effective, Excel template can be used to generate statistical frequency estimations of haplotype frequency for both single source and mixture Y-STR data from the U.S. Y-STR database.

This presentation will impact the forensic community by demonstrating how a template built using the ubiquitous Microsoft Office spreadsheet program, Excel; can be used to estimate the frequency of both Y-STR mixtures and single source casework samples. It is always desirable to estimate the frequency of a profile, or in this case a haplotype, in order to give statistical weight to a match between a crime scene sample and a known reference sample, or a more general statement of inclusion for Y-STR mixture samples. Improved use of Y-STR typing would greatly benefit the criminal justice community and the public that a laboratory serves.

In crime scene mixtures, forensic DNA typing using autosomal short tandem repeats (STRs) may not show a male component due to an excess of female DNA. Y-STR testing can be employed to visualize the male contributor to the mixture. Y-STR testing targets the male Y chromosome to generate a male-only profile. The resulting profile is termed a haplotype.

It is always desirable to estimate the frequency of a profile, or in this case a haplotype, in order to give statistical weight to a match between a crime scene sample and a known reference sample. To estimate the frequency of occurrence of a multi-locus, *autosomal* forensic DNA test, the genotype frequencies from each locus are multiplied together since each locus is inherited independently. STR loci on the Y chromosome are not inherited independently. Instead, they are transmitted as a set without re-assortment. Barring mutation, the combination of alleles on the Y chromosome will be identical to that of a male's biological father as well as other male paternal relatives. To estimate the frequency of a Y-STR haplotype, the counting method is used: the frequency is equal to the number of times a particular Y-STR haplotype appears in a database for a given population divided by the total number of haplotypes in the database.

To obtain reliable estimates a large Y-STR database is required. Several groups have pooled their databases to form the US Y-STR database (http://usystrdatabase.org/) managed by the National Center for Forensic Science (NCFS) in conjunction with the University of Central Florida. As of December 31, 2007 the US Y-STR database contained 13,906 profiles with complete, 11-locus SWGDAM-core haplotypes. The database allows profile frequencies to be estimated by users over the Internet.

It would be advantageous to provide a frequency estimate when Y-STR mixtures are obtained but currently there is no way to search the US

Y-STR Database with mixture data. We have developed a template using Microsoft Office Excel that allows the input of all alleles found in a Y-STR mixture to determine how many haplotypes from the US Y-STR database could be included in the mixture. This template will accommodate both single source and mixture samples with 10 alleles per locus, or more in scalable fashion, reporting the frequency estimates as a total and by race. Both full and partial profiles may be used for searching. The template may be used with any Y-STR typing system.

**Excel, Y-STR, Mixture Statistics**

## A133 Evaluation of the SNPlex Genotyping System for Screening Ancestry and Phenotype Informative SNPs

*Daniele S. Podini, PhD\*, Joni Johnson, BS, and Ronald Lai, BS, The George Washington University, Deparment of Forensic Sciences, 2036 H Street Northwest, Washington, DC 20052; Sabrina N. Walborn, BS, 8494 Greystone Lane, Apartment 3E, Columbia, MD 21045; and Moses S. Schanfield, PhD, Department of Forensic Sciences, George Washington University, 2036 H Street, Northwest, 102 Samson Hall, Washington, DC 20052*

The goal of this presentation is to introduce the attendees to the SNPlex™ methodology and its potential application to the screening of a high number of Ancestry Informative and Phenotype Informative Single Nucleotide Polymorphisms (AISNPs and PISNPs respectively).

This presentation will impact the forensic community by demonstrating the application of SNPs in criminal investigations. The selection of SNPs that can provide ancestry and phenotype information, combined with a high throughput cost effective method to type them, could benefit the forensic community in cases where a profile, obtained from crime scene samples, doesn't match an existing profile from a database or an identified suspect.

The objective of this presentation is to introduce the attendees to the SNPlex™ methodology and its potential application to the screening of a high number of Ancestry Informative and Phenotype Informative Single Nucleotide Polymorphisms (AISNPs and PISNPs respectively). The selection of SNPs that can provide ancestry and phenotype information, combined with a high throughput cost effective method to type them, could benefit the forensic community in cases where a profile, obtained from crime scene samples, doesn't match an existing profile from a database or an identified suspect.

Current forensic DNA testing for human identification (HID) purposes is based on the ability to generate a DNA profile from biological samples using STR markers. While STRs allow a determination of whether a sample matches an existing profile from a database or an identified suspect, the method is of limited use in solving a crime when no matches are found and no likely suspects have been identified. Further DNA analyses, targeted at inferring the phenotype and the ancestral origin of the donor, can provide information useful to the investigation by improving the ability to identify potential suspects. It is important to understand that such an assay cannot be thought of as something that will directly identify a single suspected individual but is intended as a tool to help prioritize suspect processing, corroborate witness testimony, and help determine the relevance of a piece of evidence to a crime.

The SNPlex™ Genotyping Systems (Applied Biosystems) allows the simultaneous typing of up to 48 SNPs in a single reaction. It uses an oligonucleotide ligation assay (OLA) followed by PCR, then hybridization of universal reporter probes to amplicons, and finally detection by capillary electrophoresis. The main advantages of this methodology are in the assay design, facilitated by the online tools provided by the manufacturer, and in its suitability for automation.

A review of the relevant literature on AISNPs and PISNPs was the basis for the selection of an initial battery of 60 autosomal and X-linked SNPs likely to provide information on ancestry and phenotype. For example, the Duffy (*DARC*) blood group identifies phenotypes associated with two proteins that appear on the outside of red-blood cells as a receptor; these play an important role in susceptibility to malaria infection. The Fy(a-b-) phenotype (rs2814778) represents an adaptation to living in malaria-endemic regions where mutations in the genes that produce these proteins result in this receptor not being expressed. This is a predominant feature in the African populations especially those from West Africa. Another example is the SLC24A5, a putative cation exchanger, which has been shown to be strongly involved in skin pigmentation. An A to G substitution at codon 111, which determines an Alanine to Threonine change, is a critical polymorphism within the sequence (rs1426654). The allele frequency for the Thr[111] variant ranges from 98.7 to 100% among several European-American population samples, whereas the ancestral Ala[111] allele has a frequency of 93 to 100% in African, Indigenous American, and East Asian population samples. Using the Reference Cluster ID (rs#) number all 60 SNPs were submitted to the manufacturer for assay design. Two assays were generated: one including 33 SNPs and another including 25 SNPs. Two SNPs failed the design process and were not included in either assay.

To date a total of 315 anonymous DNA samples (with self-defined ancestry), extracted from either whole blood or buccal swabs, were processed with the two SNPlex™ assays and analyzed on a 3130 Genetic Analyzer (Applied Biosystems): 80 Caucasian, 81 African American, 46 Asian, 84 Hispanic, and 24 Native American. Data were then imported into GeneMapperÒ Software V 4.0 (Applied Biosystems) and analyzed with macros specifically tailored to the SNPlex™ methodology.

In each plate 4% to 30% of the SNPs failed to meet GeneMapperÒ's default quality standard values with an average of 16.9% per plate. As a consequence the genotype at these SNPs was not called by the software for all samples on that plate. Clustering of the SNPs passed by GeneMapperÒ was consistent with the reference data. At this time modifications to the protocol are being tested to increase the number of SNPs successfully typed and a valid statistical approach to analyze the generated data is being investigated.

The SNPlex™ Genotyping Systems is not an analytical tool that can be used directly on forensic samples but rather is potentially a valuable tool for high throughput SNP screening of samples to generate reference data, although further optimization is necessary. Once a panel of the most informative AISNPs and PISNPs is identified, user friendly and sensitive assays can be developed for use in the routine crime laboratory setting.

**SNPlex™, Ancestry Informative SNPs, Phenotype Informative SNPs**

## A134 Evolution and Molecular Basis of Microvariant Alleles of the D21S11 Locus

*Megan E. Van Kirk, MFS, 310 Prettyman Drive, Apartment 7303, Rockville, MD 20850; Alexandra McCary, MFS\*, 2137 North Street, Northwest, #2, Washington, DC 20037; and Daniele S. Podini, PhD, 2036 H Street, Northwest, Samson Hall, Room 301, Department of Forensic Science, Washington, DC 20052*

The goal of this presentation is to examine the evolution and the molecular genetic basis of microvariant alleles of the D21S11 locus. Upon completion of this presentation, participants will have a better understanding of the complexity of the D21S11 repeat and its flanking regions, as well as the possible evolutionary development of the region.

This presentation will impact the forensic community because it offers a better understanding of the molecular basis of microvariant alleles and the evolution of D21S11 which is one of the most informative CODIS loci.

The CODIS locus D21S11 is a complex repeat that includes the presence of microvariants, or incomplete repeats, among certain alleles, typically those in the upper allele range. Microvariant alleles do not have a full length repeat and are therefore not an exact multiple of the original repeat pattern. These alleles are designated by the number of full repeats followed by a decimal point and number of bases in the partial unit. A complete repeat in D21S11 is a tetranucleotide, typically TCTA. The microvariant is a dinucleotide, usually presented as a TA immediately preceding the final repeat, and occasionally seen as a deletion of the TA found in the highly conserved middle section of the repeat structure. The incomplete repeat is thought to arise from single repeat gains and loses caused by replication slippage during DNA synthesis.

These microvariants are of unknown origin, but most likely arose individually by mechanistic processes (identity by state) or were fixed in an ancestral type and passed on by drift (identity by descent). The D21S11 repeat and flanking regions were sequenced in ten homozygous microvariant and non-microvariant human samples. An examination of the flanking regions of the alleles in all human samples revealed no polymorphisms, supporting the theory that the D21S11 structure is passed on through identity by descent. The internal structure of the microvariant alleles appears to have developed more recently via two separate events; one where the TA bases in the middle of the structure were lost and another where there was either an insertion of a TA immediately before the final repeat or deletion of a TC in the penultimate repeat. Although the finding had no impact on the ultimate results, complexities within the repeat region became apparent and only four of the samples were true homozygotes. The remaining six were motif heterozygotes at D21S11 but with the same overall number of bases in the repeat region.

In an attempt to understand the evolutionary process of D21S11 in humans, the repeat region was sequenced, and compared to a human reference, in four primate species: chimpanzee, gorilla, orangutan and siamang. Although the deeper molecular process is undoubtedly much more complex than what was observed in the limited number of primate sequences described here, notable polymorphisms were detected in both the flanking region and the repeat region. The analysis performed in the primate species confirmed evolutionary changes within the region. The current repeat structure at D21S11 is human specific as evidenced by mutations occurring after the evolutionary split of chimpanzee and human species. A final comparison of the human D21S11 reference sequence to the same region in a chimpanzee BLAST sequence provided a possible step-wise progression of evolution. The results of the comparison are consistent with a duplication within the repeat region of a common ancestor between human and chimpanzee that resulted in the current structure of the D21S11 locus in humans.

Due to the lack of polymorphisms in the flanking sequence among the human samples, there is no association between microvariants and a specific polymorphism in the regions contiguous to the repeat stretch of D21S11. Given this information, it is believed that the region is evolutionarily young. The D21S11 locus remains a strong genetic identifier.

**Sequencing, D21S11, Microvariant**

## A135 Principles and Applications of Fatty Acid Profiling in Microbial Forensics Investigations

*Christopher Ehrhardt, PhD\*, James M. Robertson, PhD, Vivian Chu, BS, TeeCie West, BS, and Jason Bannan, PhD, Federal Bureau of Investigation, Laboratory Division, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will be familiar with fatty acid profiling of bacteria, the effects of different growth substrates and culturing conditions on fatty acid composition of microorganisms, and the potential applications of fatty acid profiling for microbial forensic investigations. In addition, attendees will be exposed to statistical packages and techniques that can aid in the differentiation of closely related forensic samples.

This presentation will impact the forensic community by introducing novel applications of accepted microbiological techniques that can assist forensic investigators in identifying laboratory facilities and culture methods used to produce microbial bioterrorism agents.

Fatty acids are the main components of bacterial membranes that protect the cell from its environment. Cellular fatty acid profiles are determined by the genetic makeup of the organism, the nutrients available in the culturing media, and the environmental conditions present during growth. Previously, fatty acid profiling has been used for species and strain identification of unknown microbial agents in a variety of academic, industrial, and clinical settings. However, the potential for fatty acid profiles to yield forensically relevant information about the culturing conditions of microorganisms has not been explored.

In this research, three hypotheses were tested. First, can microbes grown on different media formulations be distinguished by their fatty acid profiles. Second, do changes in environmental conditions such as oxygen concentration, temperature, and pH induce significant differences in a microorganism's fatty acid profile. Third, can a post-processing statistical technique be developed that minimizes the effect of varying environmental conditions on fatty acid profiles and provide leads towards identification of media substrates that were used to grow microbes.

For this work, 12 different culture formulations were used to prepare and process sporulating cultures of *Bacillus cereus* T-strain (BcT). Fatty acid extraction and GC profiling were performed on 1-2mg of dried spore material from each media preparation using the "Instant Method" developed by MIDI, Inc. In addition, sporulating BcT cultures were grown under different oxygen concentrations, temperatures, and pH levels. The effect of media substrates and environmental conditions on spore fatty acid composition was examined using non-metric multidimensional scaling (nMDS) and Principal Component Analysis (PCA) of all generated profiles. Multivariate statistical comparisons

between each of the 12 media groups were conducted using multivariate analysis of variance (MANOVA) and Discriminant Function Analysis (DFA). The latter technique was used to generate classification functions that tested how often spores were correctly identified in their corresponding media group.

Results suggest that fatty acid profiles from spores grown on most of the surveyed media substrates can be statistically distinguished with PCA and nMDS analyses. Spores grown on Casein Acid Digest, G Media, Brain Heart Infusion, and Chemically Defined Sporulation Medium showed distinct fatty acid profiles that could be easily resolved from other media types. DFA-derived classification functions showed that almost 93% of all the spore samples (n=132) represented by all 12 media groups could be correctly identified with the growth media on which they were cultured.

In addition, changes in the oxygen concentration, temperature, and pH levels during growth and sporulation of BcT cultures caused fatty acid profile differences in certain fatty acid markers (15:0iso, 16:0, 16:1ω7c) that reduced the efficacy of spore identification with the correct media group. However, analyzing the data set with variables derived from synthesis pathways in *Bacillus* rather than individual fatty acid markers was found to minimize the effect of environmental factors and increased the likelihood that growth media for each BcT spore sample was correctly identified.

**Microbial Forensics, Fatty Acid, Statistics**

## A136    Population Studies and Proposed Nomenclature for 16 Bovine STR Loci for Forensic Purposes

*Wim A. Van Haeringen, PhD\*, and Leanne Van de Goor, MSc, Dr. Van Haeringen Laboratorium BV, Agro Buniness Park 100, Wageningen, NETHERLANDS; and Mikko T. Koskinen, PhD, Finnzymes Oy, Keilaranta 16A, Espoo, FINLAND*

After attending this presentation, attendees will understand population studies and proposed nomenclature for 16 bovine STR loci for forensic purposes.

This presentation will impact the forensic community by showing that repeat - based nomenclature is highly relevant to the field of animal forensics.

As a consequence of the close integration of cattle into the food chain of humans, forensically relevant cases involving cattle, such as identity forgery or cattle theft, are relatively common. Bovine STR loci are extensively used for parentage verification by the animal breeding industry and the first description of cattle microsatellites in the 1990s has eventually led to international recommendations for these loci in 1998 by the International Society for Animal Genetics (ISAG). While ISAG recommends certain STR loci for bovine parentage testing purposes, large scale population data reporting the information content of the loci remains scarce.

Population studies were performedon 16 polymorphic STR loci (BM1824, BM2113, ETH10, ETH225, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH3, TGLA53, BM1818, CSRM66, CSSM66, HAUT27, and ILSTS006) on 9,738 randomly selected cattle *(Bos taurus)*, representing 22 distinct breeds. The power of parental exclusion (PE 1 and PE 2), the expected heterozygosity (HE), the observed heterozygosity (HO), probability of identity (HW $P_{ID}$ and sib $P_{ID}$) as well as null allele frequencies were calculated. Here we show that, when using

microsatellite loci for forensic purposes, major differences exist in the information content of bovine genotyping between different cattle breeds. The 16 loci provide sufficient power for forensic analysis.

Furthermore, the efficacy of the loci in assigning unknown individuals to the correct breed based on genotype data, an application which has considerable use in forensic casework was studied. The individual assignment tests provided excellent success in several breeds.

For the purpose of legal casework a universal nomenclature system was established for the sixteen markers which is based on the principles of human repeat-based nomenclature according to the recommendations of International Society of Forensic Genetics (ISFG). To propose this repeat-based nomenclature in cattle, a selection of most frequent alleles was sequenced for the polymorphic dinucleotide STR loci. In the sixteen STR-markers the variable repeat structure revealed simple or compound variable nuclear tandem repeats (VNTRs); only one intermediate allele was found. The proposed nomenclature for the sixteen bovine STR markers investigated herein enabled us to successfully adopt the ISAG nomenclature to the recommendations of the ISFG for the nomenclature of human STRs.

**Bovine Short Tandem Repeats, Repeat Number-Based Nomenclature, STR Population Studies**

## A137    Genotyping Diptera Using Amplified Fragment Length Polymorphism (AFLP): Development of a Genetic Marker System for Species in the Families Calliphoridae and Sarcophagidae

*Margaret M. Wallace, PhD\*, John Jay College, Criminal Justice, Department of Sciences, 445 West 59th Street, Room 4510, New York, NY 10019; and Jason C. Beckert, BA, Microtrace LLC, 2425 Royal Boulevard, Elgin, IL 60123*

After attending this presentation, attendees will gain an understanding of the Amplified Fragment Length Polymorphism (AFLP) technique and its applicability to genyotyping non-human organisms.

This presentation will impact the forensic community by presenting a technique that can be used to genotype DNA of any origin and complexity. The AFLP method is rapid, robust, and many steps can be automated.

In forensics carrion-breeding insects are used primarily to estimate postmortem interval (PMI). Particular species are attracted to specific states of decay and colonize a body for a limited period of time. Forensic entomologists must correctly identify carrion breeding species in order to associate a particular developmental pattern with succession. This is problematic since the morphology of larvae, particularly of closely related species, is very similar if not identical. Rearing the larvae to adulthood delays the determination of PMI and may compromise the specimens by exposing the larvae to contamination, parasitism, and predation. Amplified Fragment Length Polymorphism (AFLP) is a powerful method that combines techniques from classical hybridization-based and PCR-based genotyping strategies. AFLP can be used to genotype DNAs of any origin and complexity. The AFLP technique has several advantages for forensics. The method is rapid, robust and many steps can be automated. Therefore, an identification system based on genetic markers would be a useful tool for forensic entomologists. AFLP profiles were obtained using larval samples from *Cochliomyia macellaria, Phormia regina* and *Sarcophaga bullata.* Genomic DNA was

isolated using the DNeasy Tissue Kit (Qiagen, Valencia, CA), double-digested by two restriction endonucleases (*Eco*RI and *Mse*I) and ligated to oligonucleotide adapters. Two consecutive PCR reactions (preamplification and selective amplification) were performed using a modification of the AFLP protocol described by Gibco (Invitrogen, Rockville, MD). The DNA fragments were separated by capillary electrophoresis using the CEQ 8000 DNA Fragment Analyzer. Successive selective amplifications using the *Mse*I M-CAT and *Mse*I M-CA primers produced a set of markers that, taken as whole, comprise a species specific profile. Peaks at 103, 107, 119, 127, 135, 151, 274 nt were found in the *C. macellaria* samples. The species specific profile for *S. bullata* contained peaks at 100, 102, 109, 113, 126, 128, 133, 137, 143, 165, 171, 183, 188, 242 nt. The electropherograms of the *P. regina* samples exhibited species specific peaks at 113, 131, 138, 148, 178 nt. The results indicate that the AFLP technique is a viable and valuable technique for identification of entomological material. AFLP analysis can provide answers in certain situations where traditional forensic entomology can offer no (dead larvae), or only limited (fragmented larvae), information.

**Forensic Entomology, AFLP, Genotyping**

## A138  Developmental Validation of an Improved STR Multiplex for the Forensic Analysis of Canine DNA

*Elizabeth Wictum, BS*, Teri Kun, BS, and Christina D. Lindquist, MS, One Shields Avenue, Davis, CA 95616; Nguyen Truong, BS, Veterinary Genetics Laboratory University of California, Davis, One Shields Avenue, Davis, CA 95616-8744; and Aaron Wong, BS, Center for Companion Animal Health University of California, Davis, One Shields Avenue, Davis, CA 95616-8782*

After attending this presentation, attendees will be able to acquaint those in the world of human forensic genetics with the status and potential of canine forensic genotyping. This will be accomplished through the presentation of applied research and forensic casework.

This presentation will impact the forensic community by broadening the understanding of canine forensic DNA testing and to encourage interested laboratories to consider its implementation. Expansion of crime lab capabilities to include canine genotyping has the potential to significantly impact the scope and magnitude of forensic DNA testing services and expand investigational opportunities.

The molecular analysis of biological evidence has revolutionized the criminal justice system. Its power has been used to both incriminate the guilty and free the innocent. While analysis of human DNA has been extensively vetted in courtrooms around the world, forensic analysis of non-human DNA is still gaining acceptance. To promote the admission of animal DNA evidence into the criminal justice system, practitioners must adhere to the same comprehensive validation guidelines that have been established for human DNA evidence. The Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines encourage the publication of validation studies that play an important role in the acceptance of new scientific techniques.

As the oldest domesticated species, dogs (*Canis lupus familiaris*) inhabit 39% of households in America and, after humans, are the species of greatest forensic interest. The forensic analysis of dog DNA has been the subject of several published case reports, but there have been no peer-reviewed published validation studies on the marker panels used.

Numerous genetic markers have been successfully employed to individualize canids for parentage verification, breed identification, phylogeny, and diversity assessment; however, due to the quality and quantity of DNA often encountered in forensic samples, forensic analysis requires the application of more stringent marker selection criteria. To address the lack of a standardized and validated canid forensic panel that meets those criteria, a unique opportunity was exploited to mine the recently published 7x dog genome sequence data (Broad Institute, CanFam 2.0). Publicly available markers were first masked to eliminate them from consideration. Tools were developed to query the genome for GAAA and GATA repeat motifs of 10-25 repeat lengths to identify novel tetra-nucleotide repeat markers. Over 2000 potential markers covering all thirty-eight domestic dog autosomes were identified. Candidate markers were screened for heterozygosity, polymorphic information content (PIC), probability of exclusion (PE), and ease of scoring. Fifteen unlinked highly polymorphic tetranucleotide-repeat markers were identified and assembled with the SRY sex-determination marker into a multiplex capable of generating a full DNA profile with less than 0.1 ng of nuclear DNA. To demonstrate the accuracy, precision, and reproducibility of the test, validation was carried out according to the revised SWGDAM guidelines for developmental validation. Mutation rates of 0% to 0.82% were assessed through genotyping of the expanded canine Cornell Reference family. Population statistics were generated from approximately 2500 blood and buccal samples representing both registered purebred dogs and outbred convenience samples. This panel has the potential to be not only a valuable tool for the emerging field of veterinary forensic science but also demonstrates utility for parentage verification in highly inbred dog populations and for the phylogenetic analysis of various canid populations.

**Dog, STR, Validation**

## A139  The Development and Validation of a Canine STR Reagent Kit for Use in Forensic Casework

*Mikko T. Koskinen, PhD*, Finnzymes Oy, Keilaranta 16A, Espoo, FINLAND*

After attending this presentation, attendees will be familiar with details related to development and validation of an STR reagent kit for dog and with population data generated using the reagent kit. Dog hair is commonly related to forensic casework but validated kits have so far been unavailable.

This presentation will impact the forensic community by demonstrating large-scale canine population data generated using a new reagent kit developed for forensic casework. Dog hair is commonly related to forensic casework but validated STR kits have been unavailable. Therefore, this type of potentially important evidence has been underutilized by the community. The population data will be freely available through NIST website for the forensic research community.

Biological material from pet dogs remains a largely untapped evidentiary resource in forensic investigations. The lack of well-defined STR loci, validated canine PCR/STR kits, a standardized and publically accessible database, and a well-developed nomenclature have contributed to this under-utilization.

To promote the use of domesticated dog-derived evidence, a reagent kit was developed, that enables multiplex PCR amplification of 18 STRs, and the canine sex determining Zinc Finger marker. Validation studies assessing the robustness and reliability of the reagent kit in forensic DNA

typing included sensitivity testing, reproducibility studies, intra- and inter-locus color balance studies, peak height ratio determination, characterization of artifacts such as stutter percentages and dye blobs, mixture analyses, species-specificity analyses, and case type sample analyses. The kit robustly amplified domesticated dog samples and consistently generated full 19-locus profiles from as little as 125 pg of dog genomic DNA. In addition, wolf DNA samples could be analyzed with the kit.

The reagent kit was also used in a U.S. domesticated dog population study, focussing on the capability of the multiplex in generating genetic data that sufficiently assessed the informativeness of canine forensic DNA profiles. A database comprising the raw and binned genotype data generated using this reagent kit has been uploaded onto the National Institute of Justice website (STRBase). Allele frequencies were estimated for 236 unrelated, pedigreed, and 431 mixed breed dogs residing in the U.S. Average random match probability was 1 in $2 \times 10^{33}$, using the regional database, and 1 in $4 \times 10^{39}$ using the breed dataset. Genetic diversity among and within both datasets were compared. Each pedigreed dog population was genetically distinct and could be differentiated from the mixed breed dog population. The results herein provide further support for using the allele frequency data with the canine STR reagent kit to convey the significance of identity testing for forensic casework, parentage testing, and dog breed assignment.

A nomenclature for canine-specific markers included in the reagent kit was developed based on internationally recognized recommendations for human forensic STR loci. Representative alleles were sequenced from each of the 18 STRs and the sex-typing marker included in the kit. The sequence information from the STR alleles was used to create an operationally based nomenclature. The sequence data also reflects the impact of point mutations, insertions and deletions within and outside the core repeat structures of the loci. Structural variation can affect the mobility of fragment sizing based on capillary electrophoresis, possibly confounding allele calls, allele numbers, and estimates that rely on allele frequency. Because an understanding of the STRs' sequence structure will improve the accuracy and precision of allele fragment sizing in U.S. canine forensic genetic testing, the expected allele sizes were calculated and their repeat stuctures defined based on novel allelic sequence information. Thus, it is recommend the use of an established nomenclature system.

**Canine, STR, Validation**

# A140    Watching Paint Dry:  Hypothesis Testing of Ignition Scenarios Involving Spontaneous Heating

*John J. Lentini, BA\*, Scientific Fire Analysis, LLC, 32836 Bimini Lane, Big Pine Key, FL 33043*

After attending this presentation, attendees will understand some principles of spontaneous heating and fires caused by spontaneous heating to ignition. They will be aquainted with experimental means of hypothesis testing.

This presentation will impact the forensic community by making analysts aware of situations in which simply identifying a material that is subject to self-heating is insufficient to demonstrate the cause of the fire.

In many cases, an actual experiment will be necessary. This presentation will describe experimental means for testing hypotheses involving spontaneous ignition.

In many fire investigations, it is necessary to at least rule out the possibility that spontaneous ignition was the cause of the fire. Fires in restaurants, health spas, and spray painting booths require an examination of this possibility. Most fires that occur in clothes dryers also need to be examined for the possibility of spontaneous ignition.

Spontaneous ignition occurs when material that is subject to self-heating is configured in such a way that the heat from the exothermic reaction is incapable of being dissipated to the environment. This may be due to the fact that the environment is hot, but is more often due to the configuration of the material on a cellulosic substrate. Samples of such substrates are frequently submitted to forensic laboratories for chemical analysis, and using techniques described in the literature, the analyst can derivatize the fatty acids present on the substrate and characterize them as vegetable oil residues using GC MS.[1] In the case of certain well-known hazards like linseed oil, this may be all that is necessary.

In other cases, however, it is necessary to demonstrate that under the conditions that obtained at the fire scene (the combination of the material on the substrate, the configuration of the substrate, and the ambient temperature), the package is capable of not only self-heating, but of reaching the ignition temperature of the substrate material.

Most vegetable oils are subject to self-heating at some level. The Differential Mackey test (ASTM D3523) can be used to demonstrate experimentally that a particular oil is subject to self-heating, but in most cases, no ignition will be observed. This test is generally insufficient for demonstrating that ignition can occur.

The Department of Transportation (DOT) promulgates a pair of tests, described at 49 CFR, part 173, Appendix E, designed to test the potential of materials for spontaneous heating, which is far more rigorous, and much more likely to lead to dramatic results. Although the test is designed for powders, and can be directly applied to paint overspray particles, it works well with oil-saturated towels or shop rags. If the material is capable of causing a fire in any configuration, this test will reveal that.

Thus, starting with the null hypothesis that the material in question is incapable of causing spontaneous heating to ignition, the DOT test will disprove that hypothesis if it is capable of being disproved. Once it has been shown that a material can cause spontaneous ignition, the test conditions need to be made more similar to those that obtained at the fire scene. This can result in tests that may last four days or more. Time-lapse photography and a dedicated sprinkler are useful in such situations.

Materials that include solvents (most stains and coating materials) can take an exceedingly long time to exhibit any sensible heating because the solvent absorbs the heat given off by the polymerization reaction, leading to vaporization. Only after the solvent has evaporated significantly will be temperature of the substrate increase. In some situations, it is not unusual to see the temperature increase slightly, then decline, then increase again. The reactions taking place within pile of cotton rags are anything but homogeneous, making it necessary to be very cautious with the design of the experiment and the interpretation of results.

**Reference:**

[1]    Stauffer,E, .A Review of the Analysis of Vegetable Oil Residues from Fire Debris Samples: Analytical Scheme, Interpretation of the Results, and Future Needs, J Forensic Sci, September 2006, Vol. 51, No. 5

**Fire Investigation, Spontaneous Ignition, Hypothesis Testing**

## A141 The Effect of Microbial Degradation on Ignitable Liquids

*Dee A. Turner, BS\*, and John V. Goodpaster, PhD, IUPUI Department of Chemistry and Chemical Biology, 402 North Blackford Street, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the concept of microbial degradation and how it affects fire debris analysis.

This presentation will impact the forensic community, the justice system by helping forensic chemists to identify degraded ignitable liquids.

The identification of ignitable liquids at the scene of a suspicious fire is a crucial part of an arson investigation. Since ignitable liquids are hydrocarbon based, they can provide a source of energy for microorganisms, particularly in samples containing organic matter such as soil, vegetation or rotting wood. As these microorganisms selectively metabolize hydrocarbons over time, the ignitable liquid can become difficult or even impossible to identify. This is problematic for the forensic chemist as fire debris evidence is often stored for days, weeks or even months at room temperature before it is analyzed, which provides ample time for the microbes to consume the ignitable liquid.

Research to date has demonstrated microbial degradation of ignitable liquids such as gasoline, camping fuel, barbecue starter fluid, and diesel fuel. For example, significant decreases in many of the aromatic compounds in gasoline, such as toluene and 1,2,4-trimethyl benzene was reported after 2 days.[1] After 4 days, n-paraffins and other aromatic compounds were significantly degraded.[1] However, degradation appeared to be specific to n-alkanes and lesser-substituted benzenes, as 1,3,5-trimethyl benzene and isoparaffins were not degraded even after 60 days.[1] Microbiological studies have shown that microorganisms such as *Pseudomonas fluorescens biovarIII* actively consumes aliphatic hydrocarbons, while *Pseudomonas putida*, actively consumes aromatic hydrocarbons.[2] GC/MS studies of microbial degradation in gasoline also showed preferential degradation of smaller n-alkanes and mono-substituted aromatics.[3] In addition, toluene was more degraded than xylenes, and the ratio between 3-ethyl toluene and 1,2,4-trimethyl benzene was reversed.[3]

Soil studies will be presented that have been conducted using commercially available potting soil to track the microbial degradation that is suspected to occur in fire debris. Samples of gasoline, kerosene, diesel, fuel oil #2 (dyed), odorless lighter fluid, odorless mineral spirits, paint thinner, charcoal starter, and camping fuel were all chosen for initial studies. Soil was placed inside a quart-sized paint can and 20mL of ignitable liquid was added. After the can was allowed to sit for a set time period (0, 2, 7, 14 days) at room temperature, a carbon strip was suspended into the headspace of the sample. The can was then heated in an 85°C oven for 4 hours. After cooling to room temperature, the carbon strip was extracted with 300mL pentane and vortexed for 1 minute. The resulting solution was then analyzed by GC/MS (DB-5 column, 1 mL/min helium, 1mL injection volume, 20:1 split ratio, 250°C injection temperature, Oven: 40°C for 3.00 min, 10°C/min to 280°C, 3.00 min final hold, 3.00 min MS solvent delay, scan from m/z 40-300).

In initial studies using samples of weathered gasoline, selective degradation of n-alkanes, specifically octane, decane, and dodecane, was identified after two days, with decane being the most degraded. After seven days on potting soil, the ignitable liquids were almost completely degraded. In samples of fresh gasoline, hexane, octane, decane and dodecane showed significant degradation after two to seven days, with decane and dodecane showing the fastest degradation. Also, the peak height ratio between 3-ethyl toluene and 1,2,4-trimethyl benzene reversed after two days, in both weathered gasoline and fresh gasoline. In samples of kerosene, an ignitable liquid for which microbial degradation has not been studied, preferential degradation occurred with the lighter n-alkanes ($C_{10}$-$C_{12}$). In a standard ASTM hydrocarbon mixture, the smaller n-alkanes ($C_8$-$C_{12}$) were degraded more quickly than the larger n-alkanes ($C_{14}$-$C_{18}$). Also, toluene was degraded more than p-xylene and the ethyl toluenes were not significantly degraded. Samples of ignitable liquid recovered from sterile substrates (e.g., laboratory wipes and autoclaved soil) did not exhibit degradation.

**References:**
Mann, D.C. and Gresham, W.R., **"Microbial Degradation of Gasoline in Soil,"** *Journal of Forensic Sciences,* JFSCA, Vol. 35, No. 4, July 1990, pp. 913-923.
Kirkbride, K.P., Yap, S.M., Andrews, S., Pigou, P.E., Klass, G., Dinan, A.C., and Peddie, F.L., **"Microbial Degradation of Petroleum Hydrocarbons: Implications for Arson Residue Analysis,"** *Journal of Forensic Sciences,* JFSCA, Vol. 37. No. 6, November 1992, pp. 1585-1599.
Chalmers, D., Yan, X. (Sam), Cassista, A., Hrynchuk, R., and Sandercock, P.M.L. **"Degradation of Gasoline, Barbecue Starter Fluid, and Diesel Fuel by Microbial Action in Soil,"** *Canadian Society of Forensic Science Journal*, Vol. 34. No. 2, 2001, pp. 49-62.

**Microbial Degradation, Ignitable Liquid, Fire Debris**

## A142 Factors Affecting Comparisons of Lubricating Oils

*Ryan Hibbard, BS\*, 2900 Kensington Avenue, #212, Richmond, VA 23221; and Michelle Reardon, MSFS, Bureau of ATF, 6000 Ammendale Road, Ammendale, MD 20705-1250*

After attending this presentation, attendees will be aware of factors that could potentially affect the outcome of comparisons between known and questioned lubricating oil samples. This project will discuss and evaluate the potential effect of the following factors when comparing lubricating oils: collection techniques, sample variation over time, and mixtures.

This presentation will impact the forensic community by providing valuable information to both forensic scientists and crime scene investigators. Crime scene investigators will learn a collection technique that can be used to safely collect lubricating oil samples for laboratory analysis. Forensic scientists will be presented with data that addresses possible concerns arising during comparison of known and questioned samples, such as interferences from the collection substrate, changes in a known sample over time, and the presence of fluid mixtures. This project will also benefit investigating officers, as the research will provide information regarding the association of a suspect vehicle to a lubricating oil sample present at the crime scene.

The analysis of lubricating oils can provide important information in a variety of forensic investigations such as automobile accidents and arson scenes. Forensic investigators and scientists should be aware of factors that could potentially affect the outcome of comparisons between known and questioned lubricating oil samples, so that the misinterpretation of data can be avoided. This project will discuss and

evaluate the potential affect of the following factors when comparing lubricating oils: collection techniques, sample variation over time, and mixtures.

Lubricating oils are comprised of a wide variety of hydrocarbons including alkanes, branched alkanes, cycloalkanes, and aromatics, with alkanes and branched alkanes being the principal components in the mix. As oils in an automobile undergo continued use and are exposed to high ambient temperatures, variations may be seen in the composition of the oil. Detecting these changes and determining when they occur can provide valuable information when comparing oil collected from a crime scene to oil from a suspected source vehicle.

In order to investigate collection techniques, all-purpose absorbent pads designed for clean room use, cotton swabs, sterile gauze pads, and paper towels were tested in regards to their ability to collect oil spots placed on concrete and asphalt. Oil samples were collected by rubbing the collection media over the oil spots. After collection, the oil soaked collection devices were extracted using pentane, filtered, and analyzed by high temperature gas chromatography-mass spectrometry (HTGC-MS). Blank collection substrates without oil were also analyzed by HTGC-MS to confirm that no interfering peaks were present in the chromatograms. The authors will present chromatographic data from each of the different collection media, discuss the significance of the data in regards to the effectiveness of each technique, and will address any possible interference of the concrete and asphalt in the chromatographic data.

To study potential changes over time, motor oils from eighteen different automobiles were sampled, in triplicate, from the oil crankcase dipstick of the automobiles using cotton swabs, three times over a 2 ½ month period. Extra samples were taken as necessary if an automobile received an oil change in between sample dates. Each sample was extracted from the cotton swab and analyzed using HTGC-MS. Any changes seen in the sample chromatograms will be presented, and the significance will be discussed.

Maintenance of automobiles often results in the mixing of automobile fluids (e.g., lubricating oils), which can influence the results of a comparison between known and questioned samples. Various mixtures of lubricating oils were prepared in different ratios. HTGC data for the sample mixtures will be presented and the implications of the data will be discussed.

A naturally-occurring power steering fluid (PSF) leak, from an automobile in the sample set, was used to simulate a type of lubricating oil sample that may be encountered at a crime scene. Samples were collected from the PSF leak spot on the asphalt underneath the automobile. Exemplar samples were collected from the undercarriage of the automobile where the PSF was dripping and from the PSF reservoir in the engine compartment of the automobile. The authors will introduce data that implicates the proper sampling location for exemplar samples and will address the issue of correlation between known and questioned samples.

**High Temperature GC - MS, Lubricating Oils, Motor Oils**

## A143    MS/MS Method to Differentiate Dyes in Diesel Fuel

*Lisa A. Karwacki, BS\*, 809 South Jefferson Street, #8, Allentown, PA 18103*

After attending this presentation, attendees will understand the power of MS/MS.

This presentation will impact the forensic community by explaining the development of this method allowing for the rapid analysis of diesel fuels for the presence and identification of fuel dyes.

In the United States, diesel fuels used in normal vehicle road traffic by automobiles and trucks are subject to taxation at what is termed an on-road rate. Off-road machinery using diesel fuel such as tractors, boats, farm, and logging equipment, are exempt from such taxes and as a result cost less to the consumer who purchases such fuel. Since 1994, federal and state laws have mandated the addition of red dye spectrally equivalent to 11.1 mg/L C.I. Solvent Red 26,[1] (Oil Red EGN). Such dyes are added to fuels in order to prevent their use in applications intended for higher-taxed purposes. With the current cost of fuel rising daily, people are more inclined to illegally use lower-taxed fuel in order to save money. Currently, there are two dyes that are added to off-road diesel in the United States: Solvent Red 26 and Solvent Red 164. The Environmental Protection Agency (EPA) uses the red dyes to identify high-sulfur fuels used in off-road vehicles. [2] The Internal Revenue Service (IRS) also requires the use of these dyes to identify tax-exempt diesel fuels. [3] The IRS requires that Solvent Red 164 be added at a concentration spectrally equivalent to at least 3.9 pounds of the solid dye standard solvent Red 26 per thousand barrels of diesel fuel or kerosene.

The most common method currently utilized for determining the presence of these dyes in fuels is visible spectrophotometry. This technique identifies the presence of a dye based on its absorption of light at a distinct wavelength. If significant absorption is present, a determination is made that the dye is present. This technique offers a quick and easy way to make such a determination. However, as samples become more dilute, perhaps due to the addition of higher tax fuel without the dye, the results using such means become less definitive. In such circumstances, it would be beneficial to utilize a technique to detect any dye that might be present and provides molecular confirmation in order to conclusively identify the dye specifically.

A tandem mass spectrometry method using electrospray ionization was developed to identify Solvent Red 26 and Solvent Red 164 that may be present in a diesel fuel sample submitted to the laboratory for analysis. The precursor and product ion of each compound was identified. The precursor ion for the Solvent Red 26 was m/z = 395 with product ions of m/z = 91 and 238, whereas the precursor ion for the Solvent Red 164 was m/z = 353 with product ions of m/z = 199 and 335. A multiple reaction monitoring method (MRM) was then set up to be able to monitor both dyes simultaneously in a diesel fuel sample.

Many other countries also employ the use of fuel dyes for regulatory purposes. Several other dyes were investigated. For each dye, the precursor, as well as the product ion was determined. A MRM method was set up to monitor all the different dyes tested. Each dye had a different precursor and product ion set, so each dye was successfully individualized by the method. After the completion of the method development, a blind proficiency test was completed, with each of the dyes being successfully identified. The development of this method allows for the rapid analysis of diesel fuels for the presence and identification of fuel dyes.

**References:**
U.S. code title 26, section 4082.   Code of Alabama 1975, section 40-17-22.

http://www.epa.gov/otaq/regs/fuels/diesel/diesel.htm#regs

http://www.irs.gov/businesses/small/industries/article/0,,id=98980,00.html

**Forensic Science, Fuel Dyes, Diesel Fuel**

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## A144   The Detection of Diamondoid Compounds in  Ignitable Liquid Residues by Gas Chromatography/Mass Spectrometry, Part II

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After attending this presentation, attendees will understand the importance of analyzing diamondoid compounds found in various ignitable liquid residues.

This presentation will impact the forensic community by adding compounds to analyze in ignitable liquid residues.  These compounds will increase specificity for petroleum distillates and be an aid as a possible source identification.

Various different types of petroleum products have been detected and identified during the analysis of debris recovered from suspicious fires.  Due to the complex nature of the crude oil they are derived from and the various different refining processes they are subjected to, it should be no surprise that these products contain a rich assortment of organic compounds that are useful for their classification.

For the purpose of fire debris analysis, five major classes of organic compounds have been previously identified and routinely utilized to detect and classify the types of petroleum products that may be found.  These compounds include:  (1) Normal alkanes, (2) Aromatics, (3) Cycloalkanes/naphthenes, (4) Branched alkanes/isoparaffins, and (5) Indanes.  Products encountered may differ in their content of compounds (e.g., normal alkanes predominate in distillates as opposed to aromatics in gasoline) or their ranges (e.g., light products contain more volatile components).

Recently, another class of organic compounds called diamondoids has been extensively studied in the environmental field for use in determining the origin of oil spills.  These compounds consist of rigid, three-dimensionally fused cyclohexyl alkanes.  They are naturally found in crude oils where they are formed from organic matter that decomposes around the oil.  The production of diamondoid compounds via such means results in variable production in different crude stocks.  These compounds are conserved during oil refining and, in some circumstances will become concentrated in final products.  They are rugged compounds that resist both weathering and bio-degradation.  Although they do have some industrial uses, they are rarely encountered outside of petroleum products making them very specific for the detection of and identification of such products.

Due to their rugged nature and specificity in petroleum products the addition of this class of compounds to analytical schemes in fire debris analysis would be of obvious importance.  To this end, previous research by this group has found that diamondoids, specifically adamantanes, can be found in kerosene via both liquid and passive headspace sampling.[1]

This purpose of the current work was to investigate the feasibility of extending the range of products from kerosene to include light, medium, and additional heavy petroleum products, including both distillates and specialty products.  For the purpose of detecting and identifying the compounds of interest gas chromatography/mass spectrometry (GC/MS) was performed on a range of products.  Adamantane ions were successfully extracted from chromatograms obtained from samples prepared from both liquid dilutions and passive headspace extractions.  Current research shows that the adamantanes can also be found in light, medium, and heavy petroleum distillates as well as naphthenic paraffinic products.  The diamondoids that are detected produce an easily recognizable pattern across all of the types of petroleum-based products.

Upon examination of the diamondoid profiles in different products of the same class, peak area ratios were qualitatively observed to differ.  In order to determine if these differences could be utilized for differentiation purposes, each sample was run multiple times (using both liquid dilution and passive headspace extraction), peak area ratios were calculated for all samples analyzed, and the resulting data were plotted in a three-dimensional scatter plot.  Using these data, it was found that petroleum products from the same class could be differentiated from one another.  These results suggest that adamantanes can be used as a tool to differentiate petroleum products.

**Reference:**
1.   Wert H, Brettell T, Desiderio, V.   Evaluation of the Analysis of Diamondoid Compounds *in Kerosene Residues by Gas Chromatography/Mass Spectrometry for Use in Fire Debris Analysis*. Proceedings of the 60th Anniversary Meeting of the American Academy of  Forensic Sciences, Washington DC, 2008, Abstract #**B88**.

**Arson, Fire Debris, Diamondoids**

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## A145   Discrimination of Ignitable Liquids from Matrix Interferences Using Chemometric Procedures

*Jamie M. Baerncopf, BS\*, Victoria L. McGuffin, PhD, and Ruth Waddell Smith, PhD, Michigan State University, 506 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the objective method for the discrimination of ignitable liquid residues (ILRs) from burned matrix interferences using chemometric procedures.  Pearson product moment correlation (PPMC) coefficients and principal components analysis (PCA) are used to demonstrate the discrimination of an ignitable liquid from matrix interferences as well as the association of the extracted liquid to its neat counterpart.

This presentation will impact the forensic community by providing a more objective method for analyzing ignitable liquid residues (ILRs) in forensic arson investigations, thereby minimizing the incidence of misidentification or misclassification of ILRs.

In arson investigations, ignitable liquids and ILR extracts are routinely analyzed by gas chromatography-mass spectrometry (GC-MS), and the resulting chromatograms are visually examined to identify the class of ignitable liquid present.  However, identification of the ILR is complicated by numerous factors including weathering and evaporation of the ignitable liquid during the fire, the presence of inherent

hydrocarbons in the matrix, and the presence of pyrolysis and degradation products formed during the burning process. Such interferences further complicate visual assessment of chromatograms and comparisons with reference collections of neat liquids. Because of these issues, an objective method is necessary to distinguish ignitable liquids from burned matrices and thereby minimize the risk of false positive identifications.

The purpose of this research was to develop an objective method using PPMC coefficients and PCA not only to distinguish an ILR from a burned matrix, but also to associate the ILR with its neat counterpart. The first step in this research was to explore GC-MS temperature programs to determine the fastest program that did not compromise the discrimination afforded. This temperature program was then used for all subsequent analyses by GC-MS. A reference collection of neat ignitable liquids was then compiled, consisting of ignitable liquids from six different ASTM classes: gasoline, petroleum distillates, isoparaffinic, naphthalenic paraffinic, n-alkane, and aromatic products. The ignitable liquids were extracted with activated carbon strips and the resulting extracts were analyzed by GC-MS to generate total ion chromatograms (TICs) and extracted ion profiles (EIPs) of characteristic compound classes.

The potential of PPMC coefficients and PCA for the association and discrimination of ignitable liquids from the same and different classes based on the TICs and EIPs was then investigated. PPMC coefficients were calculated to evaluate the pairwise association of ignitable liquids from the same and differing classes while PCA was used to identify natural clusters in the neat ignitable liquid data set. In the PCA scores plot, ignitable liquids from the same class were clustered closely while different classes were clustered distinctly. Loadings plots were used to determine the chemical components of the ignitable liquids that contributed the most variance to determine if other extracted ion profiles may offer increased discrimination.

A reference collection of four unburned and burned household matrices (carpet, fabric furniture upholstery, magazine, and cotton clothing) was then compiled to identify inherent hydrocarbons, pyrolysis products, and degradation products. The matrices were extracted using activated carbon strips and the resulting extracts were analyzed by GC-MS.

To examine the effects of these matrix interferences on the identification of an ignitable liquid in simulated fire debris, a set of six ignitable liquids was spiked onto each of the four matrices and burned. These spiked and burned matrices were extracted and the extracts were analyzed by GC-MS using the same procedures as previously mentioned. The chromatograms of the spiked and burned matrices were compared to those of the corresponding neat ignitable liquid and burned matrices. PPMC coefficients were calculated to assess the association and discrimination afforded between pairs of chromatograms based on the TIC and each EIP. PCA was then applied to investigate the possibility of associating the burned ILR to the corresponding neat liquid and discriminating from matrix interferences. In the burning process, the majority of the volatile components are lost, which was reflected in the TIC and some EIPs, such as the aromatic profile. Other EIPs, including the alkane EIP, were unaffected by the burning process, and hence were more useful in associating to the neat ignitable liquid, while discriminating from matrix interferences.

**Ignitable Liquids, Chemometrics, Arson**

## A146    Sustained Combustion of Bodies: Some Observations

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After attending this presentation, attendees will be able to observe combustion of human cadavers and large animal carcasses in a variety of fire exposures and learn their behavior as complex fuels. Knowing the size and duration of such fires is critical to correct interpretation of fire and medico-legal issues.

This presentation will impact the forensic community, the many forensic disciplines that may be involved in fire death investigations, and these professionals that are not familiar with fires involving bodies by familiarizing all practitioners with the qualitative and quantitative properties of such fires and reduce the likelihood of making critical errors in reconstructions or interpretations of fatal fires.

When a body is involved in a fire, it is often thought of by fire investigators as a passive target of heat and flame. In some cases, however, it becomes involved as a fuel package, contributing flames and heat of its own. It is, in rare cases, the major fuel package supporting flaming combustion in the vicinity of the body for much longer times than other fuels nearby. This paper will explore the combustion of human cadavers and similar large-animal carcasses as they burn in sustained fire environments. Previous tests have explored the thermal response and fuel characteristics in intense fires of relatively short exposure times. The tests discussed here will concentrate on fire tests where the body was the primary fuel package, but will also compare the results of fire exposures in well-fueled, well-ventilated fires in both vehicles and furnished rooms. These tests focused on long duration fires involving both intact human cadavers and torsos and whole pig carcasses of various sizes. Test fires included both fully involved vehicle and compartment fires, as well as non-accelerated, long-duration fires involving only the bedding and clothing (in the manner of typical accidental deaths where a dropped match ignites such materials).

It was observed that bodies are a complex fuel package offering several different fuels whose behavior and thermal properties vary a great deal. The subcutaneous body fat presented in nearly all bodies is, by far, the best fuel present. For it to contribute, however, the dermal layers have to shrink and split (from external fire exposure of several minutes duration), the body fat has to render out, and be absorbed by a porous, rigid substrate (often the charred remains of the bedding, clothing, upholstery, carpet or wood floor). The combustion then takes place where the body fat burns on the porous wick as a flaming fire. The size of the fire is determined by the surface area of the wick involved and the delivery rate of the fat. Fires of 20-60 kW heat output have been observed in tests where the body was the main fuel source. Given the right position of the body and wick, the flaming combustion of the body has been observed to be sustained for 4 to 8 hrs.

The limited size of the fires means that radiant heat to nearby target surfaces is insufficient to ignite them, and usually only enough to scorch or soften them, and that the air supply needed to sustain the fire is very modest. Radiant heat flux from fires involving the sustained combustion of a body has been measured in these tests to be less than 8 kW/m2. These observations mean that a sustained fire fueled by a body is capable of burning for extended periods of time without spreading to nearby fuels, unless those fuels are in direct contact with the small flames produced.

Such fires can be maintained in ordinary rooms, even with doors and windows closed. The mass loss rate of such fires is very low, measured or estimated in these tests to be on the order of 0.7 to 3 g/sec (2.6-10.8 kg/hour). With extended burn times, this could result in significant consumption of the body mass. The small flames produced are capable of desiccation, charring, and calcination of exposed bone, with eventual collapse of exposed bony structures. Muscle and collagenic components will be charred and burned away if they are exposed to the direct flames.

The results of this research will aid both fire investigators and medico-legal professionals in the correct interpretation and reconstruction of fire death scenes involving fire damage to bodies.

**Human, Bodies, Fire**

## A147 Evaluation of Different Atmospheric Pressure Ionization (API) Sources for Use in Explosives Detection

*Ching-Hong Wu, MS\*, University of Florida, 378 Maguire Village, #4, Gainesville, FL 32603; and Richard A. Yost, PhD, Department of Chemistry University of Florida, PO Box 117200, Gainesville, FL 32611*

After attending this presentation, attendees will learn of the distinct ionization mechanisms for each of these sources and which is the most efficient for applications involving explosives detection in the field.

This presentation will impact the forensic community by comparing three atmospheric pressure ionization sources and providing a basis for choosing an adequate technique for the analysis of explosives in varied scenarios and crime scenes.

This presentation will discuss the use of atmospheric pressure ionization sources for the detection of the explosives, TNT, TNB, 1,3-TNB, 2,6-DNT, Tetryl, RDX, HMX, PETN, and NG. Attendees will learn of the distinct ionization mechanisms for each of these sources and which is the most efficient for applications involving explosives detection in the field.

The development of highly sensitive techniques capable of trace explosives detection and straightforward identification is increasingly desirable in the forensic community. There is a strong demand for methods able to perform field analysis of involatile and thermally unstable explosive compounds with rapid response time, and preferably without complicated sample preparation. API is a soft ionization technique that can be operated at atmospheric pressure and room temperature, making it possible to perform mass spectral detection of explosives in the field. Three API methods, atmospheric pressure chemical ionization (APCI), desorption electrospray ionization (DESI), and distributed plasma ionization source (DPIS), were evaluated to determine ionization mechanisms and ability to detect nine explosive compounds.

The APCI has already been developed and used to detect and analyze explosives under various conditions because of user-friendliness, high sensitivity, reliability, and its widespread availability, which enables detection in ambient environment. Recently, more API sources have been developed to meet the requirements of low detection limits, high-throughput, and portability, such as DESI and DPIS. APCI uses a corona discharge at atmospheric pressure and is mainly applied to polar compounds with moderate molecular weight up to about 1500 Da and generally gives monocharged ions. The DPIS is a type of direct ionization technique for mass spectrometry which is based on the production of a nonequilibrium plasma. This plasma is generated around one of the electrodes and is fairly easy to use at atmospheric pressure to generate analyte ions. DESI is conducted under ambient conditions by spraying untreated samples with ionized solvent droplets from a pneumatically-assisted electrospray. Desorption and ionization of the analyte occurs through interaction with the charged droplet or with impacting gas-phase ions generated by the primary electrospray. These three methods, with their different ionization mechanisms, were selected because they are potentially amenable to field measurement.

In this research, 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), N-Methyl-N,2,4,6-tetranitroaniline (Tetryl), 2,6-dinitrotoluene (2,6-DNT), 1,3-dinitrobenzene (1,3-DNB), Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 1,3,5,7-Tetranitro-1,3,5,7-tetraazacyclooctane (HMX), pentaerythritol tetranitrate (PETN), and 1,2,3-propanetriol trinitrate (NG) were selected for analysis by these sources based on their structural classes: nitroaromatic, nitramine, and nitrate ester, respectively. The explosive solutions were diluted in a solvent containing 65% methanol and 35% deionized water. Experiments were performed by employing an API/MS system, comprising one of the three API sources and a commercial ion trap mass spectrometer. Gas-phase explosive ions were generated by APCI, DPIS and DESI. Negative ion mode was generally chosen for detecting the deprotonated molecule $[M- H]^-$. However, addition of an organic acid or salt is necessary to form adduct ions for nitroamine and nitrate ester explosives, such as RDX, HMX, PETN and NG, because of their lack of acidic protons. In this research, approximately 0.1% of carbon tetrachloride was used as an additive to form stable adducts ions with nitramine and nitrate ester explosives. All solutions were diluted to a concentration of about 10 parts per million (ppm), as this is fairly realistic based on calculated explosive detection applications.

**Atmospheric Pressure Ionization (API), Mass Spectrometry, Explosives**

## A148 High - Volume Dynamic Sampling Using Planar SPME Coupled With IMS for the Detection of Explosives

*Patricia Guerra, BS\*, and Jose R. Almirall, PhD, Florida International University, 11200 Southwest 8th Street CP 194, Miami, FL 33199*

After attending this presentation, attendees will understand the principles of a planar solid phase micro extraction device (SPME), a component of a complete dynamic sampling device that circulates and samples the air of large areas to pre-concentrate the volatile and semi - volatile chemical markers of explosives followed by detection using ion mobility spectrometry (IMS).

This presentation will impact the forensic community by introducing a method that could help fill the urgent need for rapidly screening cargo containers for contraband using existing ion mobility spectrometers while having other monitoring applications.

For explosives, most parent compounds have very low vapor pressure making them unavailable available in the headspace for sampling but in contrast, the chemical marker compounds associated with parent explosives are very volatile with research showing that trained canines detect these compounds instead. Pre-concentration and detection of these chemical markers using SPME-IMS has shown much success by improving detection limits over particle sampling when sampling relatively small vessels. Since the advent of the planar SPME device, that boasts larger surface area, increased capacity, and greater extraction

efficiency when compared to fiber SPME, larger areas can now be sampled for volatile and semi-volatile compounds of interest with easy coupling with existing ion mobility spectrometers by requiring no modification of the front-end of these instruments. For this research, the planar pre-concentration device is adapted to a modified handheld vacuum equipped with a filter that prevents the adsorption of unwanted particles onto the surface of the planar SPME device. Besides the analytical advantages of planar SPME over the fiber type, planar SPME is more rugged than the delicate SPME fiber for dynamic sampling at high flow rates. A controlled odor delivery device (COMPS bag) developed by Furton and workers, is used to deliver a constant rate of its contents into the ambient air. Pentalite, which is composed of 50:50 pentaerythritol tetranitrate (PETN): 2,4,6-trinitrotoluene (TNT), is used in the controlled devices, as is 2,4-dinitrotoluene (2,4-DNT), a chemical marker for smokeless powders. Since TNT is two orders of magnitude more volatile than PETN with a vapor pressure at 25°C of $7.1 \times 10^{-6}$ Torr versus $1.4 \times 10^{-8}$ Torr, it is the compound that is escaping the controlled delivery device. This is confirmed by fiber SPME-GC/MS. Two orders of magnitude more volatile than TNT is 2,4-DNT with a vapor pressure at 25°C of $2.1 \times 10^{-4}$ Torr and is also used in separate COMPS bags. The dissipation rates for TNT, a relatively volatile explosive, and 2,4-DNT a volatile chemical marker, within heat-sealed low density polyethylene bags are determined in order to expose the planar SPME device to controlled amounts of target compound. The experiments involve static extractions conducted in a sealed hood with a volume of ca. 850 cubic in. with the lone planar SPME device, and then advance to dynamic extractions using the high volume sampling device in order to take a representative sample of the total air. The sampling air flow rates are measured using a handheld anemometer, varied and then optimized. Sampling time and vessel volume are also varied and optimized. The minimum amounts of TNT and 2,4-DNT that must be present in a large volume area to produce an IMS alert are determined. The results of the static and dynamic experiments are compared. The last step is a feasibility study to evaluate planar SPME capabilities for pre-concentrating TNT and 2,4-DNT into detectable amounts by IMS following sampling of a room. The results of this study will demonstrate that high volume dynamic planar SPME air sampling followed by IMS is a promising technique for sampling high-risks area for chemical markers of explosives and even drugs.

**Planar Solid-Phase Microextraction, Ion Mobility Spectrometry, Dynamic Sampling**

## A149 Air Sampling and Detection of the Plastic Explosives C-4 and Semtex Via Their Volatile Chemical Markers by SPME-IMS

*Hanh Lai, BASc\*, Anamary Tarifa, and Jose R. Almirall, PhD, Florida International University, 11200 Southwest 8th Street, University Park Campus, CP 194, Miami, FL 33199*

After attending this presentation, attendees will understand some principles of solid phase microextraction - ion mobility spectrometry and the improved detection methodology for low vapor pressure plastic explosives.

This presentation will impact the forensic community by providing law enforcement agencies with an IMS instrumental configuration that is capable of responding to low vapor explosives via their volatile chemical markers.

This research utilizes solid phase microextraction (SPME) as an air sampling and preconcentration technique to collect the volatile chemical markers of plastic explosives C-4 and Semtex followed by detection using ion mobility spectrometry (IMS). Currently, sampling of explosives is most commonly performed via the physical removal of particles from suspected surfaces or by high volume air sampling of containers for explosive particles, following detection using analytical techniques. However, in the case of plastic explosives C-4 and Semtex, the parent compounds cyclotrimethylenetrinitramine (RDX), and pentaerythritol tetranitrate (PETN) have very low vapor pressures, making them unavailable in the headspace for air sampling. This project targets the odor signature compounds present in the headspace of the explosives, rather than the explosives themselves because these compounds are much more readily present in surrounding air due to their high vapor pressures.

The analytical instrument used in this research is a commercially available IMS fitted with a novel solid phase micro-extraction (SPME) interface previously designed in the Almirall research group. This interface allows for the desorption of SPME fibers used in the sampling and pre-concentration of volatile compounds of drugs and explosives. The IMS instrumental conditions such as drift tube's temperature, drift and carrier flow rates, reactant gas, and operating mode have been optimized systematically to simultaneously detect multiple volatile markers of the plastic explosives.

This presentation will report the odor signatures found in the headspace of explosives C-4 and Semtex using SPME-GC/MS as a confirmatory technique, and the optimized operating conditions of the IMS instrument in order to achieve the best response for the odor signature compounds, cyclohexanone, 2-E-1-hexanol, and 2,3-dimethyl, 2,3-dinitrobutane (C-4), cyclohexanol, methacrylic acid, butyl ester/ethylene ester (Semtex), as well as the IMS instrument's limit of detections and linear dynamic ranges for each of the odor signature compounds. Headspace sampling and detection of the actual C-4 and Semtex explosive mixtures will be reported. In addition, the minimum SPME extraction times and the SPME equilibrium extraction time will be reported.

**Ion Mobility Spectrometry, Solid Phase Microextraction, Plastic Explosives**

## A150 GCIR as a Tool for Analysis of Smokeless Powder Residues From IEDs

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After attending this presentation, attendees will gain an appreciation for the applicability of GCIR for the analysis of forensic samples and in particular organic constituents of smokeless powders.

This presentation will impact the forensic community by enabling the forensic analyst working in explosives analysis an additional tool in the individualization of post-blast explosive residues.

Improvised explosive devices (IEDs) are often used in domestic and foreign terrorist attacks as well as in more traditional homicides and property damage crimes. They are simple to construct with easily obtained low explosives, black powder, black powder substitutes and smokeless powders. Typically only a portion of the powder is consumed

in the explosion leaving unburned or partially burned powder at the scene. Comparison of this powder residue with samples found in the possession of a suspect can provide probative associative evidence. Traditionally comparison of morphology between smokeless powder grains has been used to narrow down to a few possible brands of powder. Chemical analysis of the extractable organic components enables the criminalist to further individualize the powder to perhaps a specific lot of powder.

Smokeless powders are mixtures of various energetic materials, plasticizers and stabilizers. Energetic materials include nitrocellulose (NC) and nitroglycerin (NG). Plasticizers, including dimethylphthalate and dibutylphthalate, are added to aid in the fabrication process, while stabilizers, such as ethyl centralite (EC) and diphenylamine (DPA) help to prevent powder decomposition during storage. DPA and EC incorporate nitrates as they are released from the propellant, forming nitrated derivatives of the stabilizers, such as N-nitrosodiphenylamine (NnDPA). Additional components such as trinitrotoluene, dinitrotoluene isomers, and camphor have been reported.

There exists a substantial body of literature on the use of capillary electrophoresis (CE), high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GCMS) for the analysis of organic components in smokeless powder. In this presentation we will present another complimentary technique for analysis, gas chromatography-infrared spectrometry (GCIR). GCIR provide another molecular identification technique which can be used to differentiate compounds which give very similar mass spectra (i.e., isomers of DNT). Comparison between GCMS and GCIR for a series of extracts from smokeless powders will be given as well as a comparison between pre-blast and post-blast powder residues.

With both GCMS and GCIR, the presence of nitroglycerine (NG) was determined without the use of 'cold on column' techniques by reducing the injection port temperature to 150°C which substantially reduced thermal decomposition of NG but did still high enough to volatilize higher boiling compounds. Acceptable resolution of all compounds (including 2,4 DNT and 2,6 DNT) was achieved with a total run time of less than 12 minutes. NnDPA was not detected by either GCMS or GCIR but was seen in HPLC and CE analyses of the same powder.

It has been found that different lots of the same powder can have very different formulations. The different formulations primarily came from manufacturers in different countries. This was especially true of the Hodgdon powders. These differences in formulations may be useful in further individualizing a forensic sample of smokeless powder. A database of organic component composition (both qualitative and quantitative) and morphological characteristics is being developed for use in establishing the statistical significance of a match between any two powder samples.

**Smokeless Powder, GCIR, GCMS**

# A151 Analysis of Trace Hydrogen Peroxide by HPLC-ED and HPLC-FD

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After attending this presentation, attendees will have be introduced to new methods for the detection of trace levels of hydrogen peroxide.

This presentation will impact the forensic community by serving the advanced level of knowledge in the field of post-blast crime scene investigation.

Recently, there have been an increased number of terrorist attacks which utilize improvised explosive mixtures. Some of these mixtures contain concentrated hydrogen peroxide mixed with a carbonaceous fuel source. When these materials are combined in the correct proportion, they can be detonated. It is therefore desirable to have methods in place which can be used to detect trace amounts of hydrogen peroxide which may be present at a post-blast crime scene.

Two methods have been developed for the analysis of hydrogen peroxide: high performance liquid chromatography with electrochemical detection (HPLC-ED) and high performance liquid chromatography with fluorescence detection (HPLC-FD). The HPLC-ED method is a direct method, meaning that hydrogen peroxide is detected as is, without further treatment of the compound. In contrast, the HPLC-FD method is an indirect method. Hydrogen peroxide is detected after interacting with a hemin enzyme and p-hydroxyphenylacetic acid in a post-column reaction. The result of this interaction is the formation of a fluorescent dimer which can readily be detected with a standard fluorescence detector. Given that neither of these HPLC methods alone permits unequivocal detection of hydrogen peroxide, concomitant use of these two analytical approaches provides a greater level of certainty.

For the HPLC-ED system, the parameters which required optimization included the flow rate and composition of the mobile phase, the column packing material, the mode of detection, the flow cell settings, and the temperature of the column and flow cell. It was theorized that optimal detection of peroxide in a matrix environment would occur when the detector was operated in pulsed amperometric detection (PAD) mode, as this mode of detection minimizes the build-up of insoluble material on the surface of the working electrode. For the HPLC-FD system, the parameters which required optimization included the flow rate and composition of the mobile phase, reagent solution, and base solution, the column packing material, and the wavelength settings of the fluorescence detector. The set-up of the HPLC-FD system was complicated, involving three pumps, three reagent solutions, and a post column reactor. Following method optimization, the linear range of the HPLC-ED system was 50 ppb to 25 ppm hydrogen peroxide. The HPLC-FD system was able to detect hydrogen peroxide over the range of 100 ppb to 5 ppm; the fluorescence detector became saturated at peroxide concentrations greater than 5 ppm. Initial studies have demonstrated that both methods are robust, with neither method readily affected by matrix components.

A limited series of field tests using improvised explosive mixtures were conducted to determine the ability of the HPLC-ED and HPLC-FD

methods to detect trace levels of hydrogen peroxide. Application of the methods to the analysis of a limited number of post-blast samples resulted in positive detection of hydrogen peroxide on post-blast material aged nine months. Additional studies will test various types of materials including metal and plastic fragments, metal witness plates, and cotton swabs. Some of these materials may be more successful at retaining trace levels of hydrogen peroxide than others; this may influence what materials/evidence will be collected by investigators at post-blast crime scenes.

**Peroxide, Explosives, HPLC**

# A152 Coming Apart at the Seams: The Anatomy of a Pipe Bomb Explosion

*Katiana M. Whitaker, Emily J. Smith, Josh N. Cummins, Benjamin J. Routon, and John V. Goodpaster, PhD*, Indiana University Purdue University Indianapolis (IUPUI), Forensic and Investigative Sciences Program, 402 North Blackford Street, LD 326, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the process by which pipe bombs explode, the effects of container type and explosive filler on fragmentation, as well as the mass distribution and velocity of the resultant fragments.

This presentation will impact the forensic community by providing forensic examiners with guidelines for interpreting pipe bomb blast effects as well as an appreciation of the potential lethality of pipe bomb fragments.

Pipe bombs are one of the most common types of improvised explosive devices encountered in the United States. The interpretation of pipe bomb blast effects can often lead to crucial information regarding the type of container as well as explosive filler used. For example, steel pipe bombs containing black powder or black powder substitutes such as Hodgdon Pyrodex® will produce few large fragments and the pipe may split at its seam. The end cap face plates are often blown out and fragments will exhibit square, 90° edges. Heavy grey or black residue will be present on the interior surfaces of the pipe, sometimes with a "rotten egg" smell. Finally, the pipe may be rusted due to the formation of corrosive by-products. In contrast, pipe bombs containing double-base smokeless powder (DBSP) such as Alliant Red Dot® will have no apparent residue and the interior surfaces of the pipe may even be "shiny". There will be extensive fragmentation, including 90° breaks as well as 45° reversing slants on edges. Finally, the pipe fragments may be thinned due to the force of the explosion. These observations, although based on the extensive experience of forensic chemists, have not been fully studied in a quantitative fashion. The lethality of pipe bomb fragments is also not fully appreciated and the velocity and momentum with which container fragments leave the site of an explosion is not well known.

The goals of this project were to compare the effects of container material and explosive fill on pipe fragmentation. A total of seven devices were constructed from 1-inch nominal diameter galvanized steel, black steel and PVC pipe with either Pyrodex® or DBSP filler. All devices were suspended in open air and initiated with electric matches. Container fragments were gathered and examined for morphology, mass distribution and explosive residue. The mass distribution of the container fragments was evaluated using the slope of the Fragment Weight Distribution Map (FWDM).[1] In this approach, steep slopes correspond to the production of many small fragments, whereas shallow slopes correspond to the production of fewer larger fragments. Video footage of the pipe bomb explosions was also captured using a high-speed digital video camera with telephoto lens at astandoff distance of ~60 feet. Videos were shot at 10,000 frames per second (100 µs/frame) with a 1/51,000 second (19.6 µs) shutter speed. Analysis of this footage revealed the locations where the pipe containers first failed as well as provided estimates for the velocity of expelled fragments.

The distribution of fragment masses for all devices was approximately exponential. However, PVC pipes generated larger numbers of smaller fragments. For example, over 75% of the fragments from PVC pipe filled with DBSP had individual masses less than 300 mg, with each representing only a tiny faction (< 0.3%) of the total mass of all recovered material. In addition, the initial slope of the FWDM for devices filled with DBSP showed a clear difference between PVC (m= -61.5) and either black steel (m= -2.9) or galvanized steel (m= -2.5). The high-speed video footage of the pipe bomb explosions also shows a clear difference between devices consisting of PVC pipe versus steel pipe. Devices made from PVC pipe first ruptured along the pipe nipple itself, regardless of explosive filler. Devices made from steel pipe first ruptured at the end caps. The estimated velocity of the container fragments also varied depending on their origin. For example, the estimated velocity of a fragment originating from a PVC pipe nipple filled with Pyrodex was 465 mph. Similarly, the estimated velocities of fragments originating from a PVC pipe nipple filled with DBSP ranged from 252 mph to 469 mph. In contrast, the estimated velocity of a fragment originating from the end cap of a PVC/Pyrodex device was only 86 mph. Overall, the highest estimated fragment velocities originated from the galvanized steel/DBSP (351 mph – 476 mph) and black steel/DBSP (291 mph – 556 mph) devices.

**Reference:**

1. Oxley, J.C., et al., *Improvised Explosive Devices: Pipe Bombs.* Journal of Forensic Sciences, 2001. **46**(3): p. 510-534.

**Explosives, Pipe Bomb, Improvised Explosive Device**

# A153 Forensic Discrimination of Red Hair Dyes by UV-Visible Microscpectrophotometry

*Jay A. Siegel, PhD*, John V. Goodpaster, PhD, and Julie Barrett, MS, Indiana University Purdue University Indianapolis, School of Science, LD 326, 402 North Blackford Street, Indianapolis, IN 46202*

After attending this presentation, attendees will appreciate the value of cosmetic hair dyes in the analysis of human hairs and will learn how UV microspectrophotomerty is used in characterization of hair dyes.

This presentation will impact the forensic community by showing that hair dyes can be valuable evidence in the characterization of human hairs as evidence in crime scenes and how it can strengthen the association of hairs to individuals.

Human hairs occur in a wide variety of crimes, especially those involving violence. Hairs are easily shed and transferred from one surface to another. In recent years, DNA typing of the hair root and mitochondrial DNA typing of the hair body have added specificity to the analysis of hair and have provided a possible means of individualization in some cases. However, little attention has been paid to the analysis of cosmetic hair dyes that are often found in hair. The aim of this project is to successfully discriminate between hairs dyed with different commercial and professional red dyes using UV-Visible Microspectrophotometry, as well as to evaluate the proposed method as a

viable approach to analyzing hair dyes as supplemental evidence in forensic hair examinations. The morphology and microscopic features of human hair provide a wealth of information such as species of origin, area of the body from which the hair originated, ethnicity, method of removal from the body, either forcible or naturally shed, in addition to disease states, thermal damage, and cosmetic modifications. Although cosmetic modifications occur with significant frequency, such as hair bleaching and/or dyeing, insufficient research has been performed in order to further distinguish and identify the products responsible for these modifications. In this project, a comprehensive set of fifty-five professional and consumer red hair dyes was analyzed with UV-Visible Microspectrophotometry and evaluated using multivariate statistical techniques including Agglomerative Hierarchical Clustering, Principal Component Analysis, and Linear Discriminant Analysis. The dyes were grouped into three classes, consistent with macroscopic visual inspection, yielding a classification accuracy of 81.45%. An external validation was performed by collecting new data for twenty of the dyes, resulting in a prediction accuracy of 76%. Three single-blind trials were also conducted, with two correct classifications and one inconclusive result. Temporal stability testing demonstrated consistent spectra throughout the duration of the study, spanning five weeks. Estimated fading of the dyes with successive washing indicated that significant color loss is apparent within three weeks of dye application. Finally, reduced classification accuracy was observed for calculated first derivative spectra relative to original data for a subset of fourteen professional hair dyes. The results showed that UV-visible microspectrophotometry can be a valuable technique in distinguishing among dyed hairs. It can provide useful, additional information about the association of hair with an individual beyond morphological examination.

**Hair Analysis, Hair Dyes, Microspectrophotometry**

## A154  Optical Characterization of New "Eco-Friendly" Fibers

Kelly M. Brinsko, MS*, McCrone Research Institute, 2820 South Michigan Avenue, Chicago, IL 60616

After attending this presentation, attendees will have an understanding of the optical properties of the new azlon (protein) fibers, polylactic acid fibers, and others that are touted as being ecologically friendly.

This presentation will impact the forensic community by alerting analysts to these new fibers which are becoming increasingly popular to the environmentally conscious public.

With the recent focus on environmentally friendly products, manufacturers have begun producing fibers to meet the growing demand of an increasingly earth-conscious public. These fibers include azlon (regenerated protein) fibers, polylactic acid fibers, and bamboo rayon. Because these fibers are made from naturally occurring polymers of corn, milk, soybeans, bamboo, and others, they are touted by manufacturers as biodegradable and "eco-friendly", and an alternative to petroleum-based fibers such as polyester. As they become more popular, these fibers will begin turning up in forensic casework. It is important that forensic scientists are aware of these new fibers and are able to recognize them when they are encountered.

While azlon fibers experienced a boom in the 1940s and 1950s, they were discontinued in the United States during the 1960s. However, azlon production is reemerging in other parts of the world and is available to consumers in the form of yarns and textiles. Optical characterization in the literature is sparse, and older data does not seem to be accurate in relation to today's azlons. Azlon can be produced from proteins in corn, milk, and soybeans. The optical characteristics can vary depending on the raw materials or method of manufacture.

Polylactic acid (PLA) fibers are produced by polymerizing dextrose from starch, usually corn starch. This fiber is currently being mass-produced by manufacturers in the U.S. and Japan, and is used both in textiles and in medical applications such as sutures. PLA fibers have caught the attention of the forensic science community, and several papers have been published on their optical qualities.

Because of the extremely fast growth rate of bamboo, it is considered a renewable resource, and is currently used in a variety of consumer products including furniture and flooring. The bamboo rayon fiber is produced in much the same way as viscose rayon, and is optically similar. An attempt has been made by one company to differentiate bamboo rayon from viscose, and this method will be examined here.

This presentation will show polarized light observations, including refractive index and birefringence, as well as hotstage melting temperature. The characterization also includes FTIR spectra, cross-sectional shapes, and solubility determinations. Identifying characteristics of the fibers, as well as a method of distinguishing them from the more common fiber types will be presented.

**Fibers, Azlon, Polylactic Acid**

## A155  The Evaluation of Human Hand Odor Volatiles on Various Fiber Chemistries: A Comparison Between Contact and Non-Contact Sampling Methodologies

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After attending this presentation, attendees will learn the basic concepts of the effect of fiber chemistry and collection protocol on collected hand odor volatiles.

This presentation will impact the forensic community by presenting the development of an optimized collection protocol which helps clarify the instrumental definition of an individual odor sample, and also helps develop a more standardized methodology for law enforcement personnel to implement when acquiring scent evidence in the field.

The use of human scent as a type of forensic evidence for legal proceedings lies in the idea that human odor is a unique physical characteristic of every individual and that this odor is left at every location, object, or path which the subject has come in to contact with. Research in the area has focused on an instrumental definition of what constitutes human odor comparing and contrasting target volatiles emanating from humans from different body regions as well as different collection procedures. Furthermore, human scent discriminating canines have offered a practical application of scent evidence by utilizing it as their target source when tracing the location of a person or suspect of a crime thus indicating possible scent matches.

Law enforcement and laboratory personnel have utilized both contact and non-contact approaches for the efficient collection of human

scent samples. To date, however, there is no optimized collection protocol for efficient trapping capabilities of chemical profiles. There are two main possibilities for the efficient collection of human scent samples, a contact and non-contact approach. Contact methods include the direct contact with an absorber medium by the suspect or direct swiping of body regions with a sterile gauze pad and transferring scent from the subject to the collection medium. The non-contact methods of human scent collection include a direct absorption of scent by placing a sterile gauze pad near the subject for an unspecified amount of time or utilizing the Scent Transfer Unit (STU-100) as a collection device. As such, collection procedures have not been fully compared for efficient and optimized results. The newly developed Scent Transfer Unit allows for the ability to perform non-contact scent collection using dynamic airflow from objects or suspects without contaminating or altering the object/target of interest, however, chemical profiles obtained from human subjects has not been evaluated with the device. Furthermore, there is a lack of research as to the most efficient absorber medium utilized in laboratory and field work applications.

The focus of the present study is to evaluate and compare both contact and non-contact human scent collection procedures utilizing an array of different absorber mediums to understand the chemical composition of human hand odor volatiles on various fiber chemistries. The materials evaluated in this study were selected to include a range of both natural and synthetic fiber chemistries from which to optimize the best collection medium for odors from forensic specimens. The studies included an instrumental analysis using headspace solid phase micro-extraction in combination with gas chromatography / mass spectrometry (SPME-GC/MS) for the study of hand odor collected utilizing the Scent Transfer Unit over the palms of the hands of both female and male subjects for a period of 1 minute samplings as well as a parallel study with the varying absorbent materials to evaluate the direct collection of human hand odor samples. A comparison of the profiles obtained in the contact method vs. non-contact approach was conducted in order to establish the reproducibility and similarity of both chemical profiles from the individuals evaluated. The contact approach consisted of a hand odor sample procedure in which subjects were asked to hold the materials between the palms of their hands instead of a close proximity. Ongoing research has shown that fiber chemistries such as cotton yield higher mass amounts of collected scent than non-polar fiber backbones such as polyester; thereby showing differences in chemical profiles depending on the collection fiber chemistry.

The development of an optimized collection protocol helps clarify the instrumental definition of an individual odor sample, and also helps develop a more standardized methodology for law enforcement personnel to implement when acquiring scent evidence in the field. Both sampling methods were used on the same individuals thereby providing the scientific community with laboratory data exhibiting the usefulness or disadvantages of implementing each method as well as an informed selection of absorber mediums for enhanced scent profiling.

**Scent Transfer Unit (STU-100), Absorber Medium, Contact and Non-Contact Methods**

## A156 Significance of Match Criteria for Refractive Index Comparison of Glass Fragments

*Robert D. Koons, PhD\*, CFSRU, FBI Academy, Quantico, VA 22135; and Elizabeth J. Garvin, BS, 17620 Old Jamestown Road, Florissant, MO 63034*

After attending this presentation, attendees will obtain an appreciation of the relationship between commonly used match criteria for refractive index of glass and the frequency at which a recovered fragment or fragments are incorrectly not matched with their true source for typical panes of float glass.

This presentation will impact the forensic community by discussing how the majority of forensic laboratories currently use some form of hypothesis testing for comparison of RI between samples. In most instances, a confidence interval about a central value is determined by repeated measures of a sample of known origin (here referred to as K) and values measured for recovered fragments (Q), either individually or grouped, are compared to this interval. Common statistical tests are used for this comparison, sometimes without consideration of the underlying requirements or assumptions of the test. There is currently a high level of interest in defining a standardized procedure for these match criteria that can be used by a number of forensic practitioners. In a study to be discussed in this presentation, repetitive random selection of measurements from each of five sheets of float glass were used to assess eight match criteria as to the frequency in which a fragment fails to be associated with its correct source.

Five glass sources were utilized for this study, two sheets of a double paned residential window, one automobile side window, and two sheets of a laminated automobile windshield. All five samples were float glass. The automobile side window was tempered and the other four samples were not. Sample fragments were selected from each of the four quadrants of each sheet. From within each quadrant, five fragments were selected, washed with nitric acid, crushed, and mounted in silicone oil (Oil B, Locke Scientific) on slides for measurement of refractive index. For each prepared slide, at least ten measurements of RI were obtained at the 589-nm wavelength ($n_D$) using an automated glass refractive index measurement instrument (GRIM3, Foster and Freeman). The first ten edges that were of good quality were selected for measurement. Edges corresponding to the original glass surface were avoided and no two measurements were made from the same edge of a fragment. Using this procedure, at least 200 measurements were made from scattered locations throughout each glass source. Prior to making analytical measurements, the instrument was calibrated using seven glass standards (B Series, Locke Scientific) to obtain a linear response between $n_D$ values at 20C and the match temperature. Accuracy of the results was checked daily using a reference glass standard material (NBS 9012).

The 200+ results for each source were used to select data representing K and Q glass fragments for evaluation of eight match criteria that have been used in forensic laboratories. The match criteria tested consisted of confidence intervals based on range, range $\pm0.00005$, mean $\pm1$ standard deviation, mean $\pm2$ standard deviations, t-test at 95% confidence, t-test at 99% confidence, mean $\pm0.0001$, and mean $\pm0.0002$ (Miller criterion). Each test was performed 1000 times for each glass source by random selection with replacement from the measured values. The effects of the number of measurements were determined by performing each statistical test at a number of recovered glass measurements of 5, 6, 7, 8….20, 25, 30, and 40 and the number of

questioned fragments of 1 and 3, i.e., 19 levels of K by 2 levels of Q by eight statistical tests, repeated 1000 times.

The error rates differ from those that might be predicted by statistics for some tests, probably as a result of minor deviations from normality in the distribution of the measured values. Predictably, the number of replicate measurements on the K sample has a significant effect on the error rate for some of the tests. Also, when three measurements of the Q glass are averaged together and compared to the K glass, the number of Type 1 errors is smaller than when only a single measurement of the Q glass is used.

**Glass, Refractive Index, Match Criteria**

## A157 Discrimination of Glass Samples by Infrared Microprobe Analysis With Diamond Attenuated Total Reflection

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After attending this presentation, attendees will learn how infrared microprobe analysis can be a useful tool for forensic glass analysis.

This presentation will impact the forensic community by demonstrating a new method for discriminating between various types of glass samples, and illustrating the significance of using infrared microspectroscopy.

Glass is a component in numerous everyday objects, from bottles and containers, to windows and laboratory glassware. It is an excellent source of physical evidence because of its stability as well as ability to be transferred easily, making it commonly encountered as fragments due to its susceptibility to breakage. Because of these characteristics, the importance of reliable comparative analysis therefore cannot be understated, especially as glass can be found in both civil and criminal investigations. Over the last few years, glass examination has gradually moved towards elemental analysis and the identification of elemental composition, using ICP-MS and LA-ICP-MS. However, there is still a need for more discriminatory methods for forensic analysis, and current research with infrared microspectroscopy of glass shows great potential.

This research focuses on the analysis of several glass samples from the National Institute of Standards and Technology (NIST), for which compositional data is available, using infrared microspectroscopy with diamond attenuated total reflection (ATR). Infrared microprobe analysis (IMA) combines microscopy with IR spectroscopy, allowing for both visual examination and midinfrared spectral analysis of a material. IMA of glass is advantageous because it has the potential to provide chemical functional group information from the spectrum of the glass. This information from the spectral data in combination with the compositional data for the glasses allows for better comparisons.

The infrared microprobe is quick and simple to use, requires little to no sample prep, and is generally non-destructive. The Attenuated Total Reflection objective (ATR), which focuses the IR beam on to the diamond-glass interface, allows for ATR spectral measurements. The generated ATR spectrum can then be analyzed using various software programs and searched through various ATR spectral data libraries for comparison. Several spectra were obtained for each sample and used to create an ATR library. Each spectrum was then run in the library first against spectra from the same sample in order to test reproducibility, and

then against all other spectra in order to test the discriminating power of this method.

The results show that despite fact that the elemental chemistry of the NIST samples used is very similar, it was still possible to observe the differences between the samples and distinguish between them. Overall, the success rate for identifying the correct number 1 hit in the library searches was an overwhelming 96.49%, with the correct answer always being within the first two hits, proving the usefulness of this method. In addition, this research emphasizes the need for the development of specialized spectral databases, such as ATR spectra, which can be invaluable for forensic research.

**Glass, Infrared Microspectroscopy, Trace Evidence**

## A158 Accredition of a Forensic Case Work Method Using Laser Ablation ICP-MS for the Examination of Glass According DIN EN ISO/IEC 1705 and Implementation of Interpretation and Reporting According DIN EN ISO/IEC 17020

*Stefan Becker, Dr.\*, Bundeskriminalamt, Forensic Science Institute, KT 13 Bundeskriminalamt, Thaer Strasse 11, Wiesbaden, 65173, GERMANY*

After attending this presentation, attendees will have an inside view of a particular German approach to ensure a certain level of transparency and quality in reporting trace evidence cases.

This presentation will impact the forensic community by presenting new trends in the accreditation process in Europe/Germany.

Introduction of a routine forensic case work LA-ICP-MS method for the examination of glass according DIN EN ISO/IEC 17025 and implementation of interpretation and reporting of case work according DIN EN ISO/IEC 17020

With the beginning of the 1990ies, first quality management activities in the field of European forensic science services were started. Several European forensic science institutes received accreditation (e.g., Great Britain, Sweden, and the Netherlands).

On a European level ENFSI (European Network of Forensic Science Institutes) requests that all member laboratories should have achieved or should be taking steps towards ISO/IEC 17025 compliant accreditation for their laboratory testing activities.[1] .

Due to these external circumstances the direction of BKA decided that accreditation of the forensic science institute was favourable. Based on a decision made in 2003 accreditation of the forensic science institute according ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories" was put forward. In 2006 the first 6 out of 20 units of the forensic science institute were accredited according to the norm DIN EN ISO/IEC 17025.

In September 2007 the inorganic material analysis section (KT 13) of the Bundeskriminalamt /Germany received accreditation (ISO/IEC 17025:2005) for methods in the field of glass and paint examination including the operation of the European Collection of Automotive Paints (EUCAP). In the field of glass analysis the main methods involved were the determination of refractive index and the elemental quantification of glass fragments applying laser ablation inductively plasma mass spectrometry (LA-ICP-MS).

Late 2007 it was decided that all units of the Forensic Science Institute of the Bundeskriminalamt that already achieved accreditation

according DIN EN ISO/IEC 17025:2005 should additionally undergo accreditation according DIN EN ISO/IEC 17020:2004 "General criteria for the operation of various types of bodies performing inspection". This should be achieved until October 2008. By this step the complete forensic process including case work reporting should be covered by the so called integrated quality management system.

Further activities of KT 13 lead to a successful accreditation audit of the entire process of interpretation and reporting of glass case work according DIN EN ISO/IEC 17020 in October 2008. Accreditation is expected to be received at the end of this year.

The different phases of the accreditation processes - testing of the analytical methods, method validation, and application of a conclusion scale - will be presented.

The validation and the definition of match criteria for the comparison of quantitative results by LA-ICP-MS analysis of glasses based on the statistical evaluation of type I and type II errors is described briefly.

The conclusion scale based on five levels of material association types is described. The application of this type of conclusion scale for the use in paint case work reporting has already been presented previously.[2] The material specific application (glass/paint) will be highlighted in the presentation. Glass case work examples will be given.

**References:**
1   ENFSI policy document QCC-ACR-001 "Standards for accreditation" 2007
2   Bommarito C. oral presentation at SWGMAT meeting May 2008, Fredericksburg/VA
**LA-ICP-MS, Accreditation, Glass**

## A159   The Application of Cathodoluminescence to the Analysis and Comparison of Concrete Block Samples

*Dyanne E. Cooper, BS\*, 1564 Barry Drive, North Huntingdon, PA 15642; Graham F. Peaslee, PhD, Hope College, Chemistry Department, 35 East 12th Street, Holland, MI 49423; and JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, FBI Academy, Building 12, Quantico, VA 22135*

After attending this presentation, attendees will understand the nature and components of concrete and how it is manufactured, the characteristics of minerals that allow them to become luminescent when bombarded with electrons, and how cathodoluminescence can be used with digital imaging to differentiate concrete samples.

This presentation will impact the forensic community by providing a semi-automated method for the collection and interpretation of cathodoluminescence and digital imaging of concrete materials. The purpose of this project is to develop a method using cold cathodoluminescence (CL) in conjunction with reflected light (RL) microscopy and image processing to discriminate among various sources of concrete (e.g., manufacturer, location, production batch).

The purpose of this project is to develop a method using cold cathodoluminescence (CL) in conjunction with reflected light (RL) microscopy and image processing to discriminate among various sources of concrete (e.g., manufacturer, location, production batch). This presentation will impact the forensic community by providing a semi-automated method for the collection and interpretation of cathodoluminescence and digital imaging of concrete materials.

Concrete is a mixture of cement, water, and aggregates, where the aggregates are typically the major component of the mixture. Concrete is reported to be the most ubiquitous manmade substance on Earth and, as such, it is often found in connection with criminal activity. The components of concrete – cement, water, and aggregates – contain certain kinds of minerals, such as quartzes, carbonates, and feldspars. Many of these minerals contain impurities known as luminescence activators and lattice defects that allow the crystal to undergo both intrinsic and extrinsic luminescence when excited by an electron source. This luminescence is due to not only the mineral itself but also the presence of sensitizers and/or quenchers in its surroundings. This visible luminescence can be recorded via a digital camera or a UV-Vis-NIR spectrometer, both of which can be attached to the trinocular head of a microscope.

In this project, reflected light (RL) and cold cathodoluminescence (CL) image collection is combined to differentiate concrete samples that are difficult to distinguish with the naked eye. RL microscopy allows for both low- and high-magnification images to be collected and processed for color gates using image processing software. Processing CL images for color gates adds an extra investigative tool to differentiate samples that appear similar through RL analysis. Using a combination of image processing and statistical methods, the RL and CL data can be interpreted in numerical and graphical form to provide an objective basis for differentiation of concrete materials.

**Concrete, Cathodoluminescence, Minerals**

## A160   The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

*Scott R. Oulton, BS\*, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

After attending this presentation, attendees will understand the current status of SWGDRUG's work products will be discussed. Representatives from the SWGDRUG Core Committee will answer questions and address the concerns of attendees.

This presentation will impact the forensic community and/ or humanity by providing the current work products by SWGDRUG as it relates to the analysis of seized drugs.

The objective of this presentation is to update forensic drug analysts on recent work products from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). Currently, SWGDRUG is working on the following topics:

- Uncertainty in measurements
- Reporting protocols (what should the report say?)

These topics have been widely discussed in the forensic science community. During this presentation, the current status of SWGDRUG's work products will be discussed. Representatives from the SWGDRUG Core Committee will answer questions and address the concerns of attendees.

In past presentations to the American Academy of Forensic Sciences, a synopsis of the history of SWGDRUG and goals of the core committee have been presented. This year's presentation will focus on the specifics described above. However, the following information is presented here for those unfamiliar with the SWGDRUG process. SWGDRUG has been in existence since 1997. The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance.

The objectives of SWGDRUG are the following:

- Specifying requirements for forensic drug practitioners knowledge, skill and abilities,
- Promoting professional development,
- Providing a means of information exchange within the forensic science community,
- Promoting ethical standards of practitioners,
- Providing minimum standards for drug examinations and reporting,
- Establishing quality assurance requirements,
- Considering relevant international standards and
- Seeking international acceptance of SWGDRUG recommendations

The SWGDRUG core committee is comprised of representatives from federal, state and local law enforcement agencies in the United States, Canada, Great Britain, Germany, Japan, Australia, the European Network of Forensic Science Institutes (ENFSI), the United Nations Drug Control Program (UNDCP), forensic science educators, the American Society of Crime Laboratory Directors (ASCLD), ASTM, and the National Institute of Standards and Technology (NIST). Published recommendations are available on the SWGDRUG website.

**Analysis of Drugs, SWGDRUG, Seized Drugs**

## A161 Anion Identification Via Complexation With Meso-Octamethylcalix(4)pyrrole and Electrospray Ionization Mass Spectrometry (ESI-MS) Detection

*Sandra E. Rodriguez-Cruz, PhD, and Kathryn Carson, BS\*, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

After attending this presentation, attendees will become familiar with a new method for the identification of counterions using the anion-binding agent meso-octamethylcalix(4)pyrrole and electrospray ionization mass spectrometry (ESI-MS) analysis.

This presentation will impact the forensic community by demonstrating a new technique to identify salt forms in forensic drug analysis.

Salt form identification has become routine in forensic drug analysis, as it could result in differences in sentencing based on salt form (i.e., cocaine HCl vs. cocaine base), and can also identify counterfeit pharmaceuticals. Currently acceptable anion identification techniques include precipitation tests, such as the $AgNO_3$ test, and infrared spectroscopy (IR). While precipitation tests can distinguish between halides, many other salt forms do not produce distinct results. IR spectra of different salt forms are not always distinguishable from one another, as is often observed in the analysis of black tar heroin. Nuclear magnetic resonance (NMR) results may also distinguish between the base form of a substance and a salt form, but specific identification of the anion is not possible.

The compound *meso*-octamethylcalix(4)pyrrole (C4P) is a member of a class of functionalized calix(4)pyrroles that have been the subject of recent research. It has been found that this class of molecules non-covalently coordinates to anions, producing a large ion with an overall negative charge. The soft ionization conditions of ESI-MS allow the complex to be transferred intact into the gas phase and subsequently into the MS detector, allowing collection of molecular weight data.

Subtracting the weight of the C4P (MW=428) gives the molecular weight of the anion.

A method for the identification of basic drug anions has been developed using a quadrupole ion-trap mass spectrometer with an ESI source. Solutions were prepared in acetonitrile at concentrations of 10-15 ug/ml and injected directly into the mass spectrometer via a syringe pump. Complex formation was achieved by the addition of 50 ul of a 0.5 mg/ml solution of C4P in acetonitrile to 1 ml of sample solution. Data was collected in both positive and negative ion modes for each sample; analysis of each sample was complete in two minutes or less.

Complexation with C4P was observed for chloride, bromide, iodide, acetate, and nitrate salts. Expected isotope ratios corresponding to chlorine and bromine were observed, providing further confirmation of formation of the anion-bound complex. Anion complexation was not observed for sulfate or phosphate salts. Anion selectivity tests were also performed by testing solutions with multiple anions in equimolar amounts. In accordance with other papers on the issue, chloride ions seemed to have a higher binding affinity to C4P than bromide ions.

It was determined that complexation of a sample with C4P and subsequent analysis by ESI-MS is a viable and rapid means for identification of many common anions found in forensic drug samples.

**Electrospray Ionization, Forensic Drug Analysis, Anions**

## A162 Rapid Analysis of Multiple - Unit Exhibits Using Mass Spectrometry: No Chromatography Necessary

*Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

After attending this presentation, attendees will have knowledge of the recently developed techniques of ESI, APCI and DESI, and their used during forensic chemistry casework.

This presentation will impact the forensic community by increasing awareness regarding new state-of-the-art analytical techniques of great used to the criminalistics community.

The development of the soft ionization technique of electrospray ionization (ESI) during the mid eighties extended the application of mass spectrometric (MS) techniques to the analysis of large, polar, non-volatile molecules. During the last 20 years, applications of ESI and other atmospheric pressure ionization (API) techniques have exploded and these days the use of LC-MS instrumentation is relatively common in academia and the pharmaceutical industry. However, the adaptation and utilization of these techniques in forensic laboratories has been slower, as the traditional technique of choice for MS analysis is usually gas chromatography–mass spectrometry (GC-MS). In this presentation, the advantages and limitations of ESI, atmospheric pressure chemical ionization (APCI) and desorption electrospray ionization (DESI) will be discussed. Examples will be presented demonstrating the successful use of ESI-MS/MS, APCI-MS/MS and DESI-MS/MS experiments during the screening of multiple-unit exhibits.

ESI and APCI are the two most common ionization interfaces used during routine liquid chromatography - mass spectrometry (LC-MS) experiments. During ESI, ions in solution are transported into the gas phase via a series of solvent evaporation and Coulomb explosion events. Greatly influenced by solution chemistry properties, the softness of the ESI process allows the detection of intact compounds as singly or multiply protonated ions of the form $(M+nH^+)^{n+}$. For APCI, ionization

solely occurs via single protonation of the analyte based on its gas-phase chemistry. Both ionization techniques, when interfaced to a mass spectrometer, provide molecular weight information and allow the use of collision induced dissociation (CID) experiments for structural elucidation. Ionization via ESI is ideal for the analysis of polar controlled substances, while APCI is more amenable for the study of compounds of intermediate or low polarity, like anabolic steroids and cannabinoids.

DESI is one of the most recently developed ambient ionization techniques, where samples can be analyzed either directly or after deposition onto a non-conducting surface. An extension of electrospray, DESI allows the rapid screening of tablets, liquids, and plant material without the need for sample preparatory steps. Analytes are also detected as protonated species providing molecular weight and structure information via MS experiments.

In this presentation, presumptive and confirmatory MS methods using ESI, APCI and DESI will be presented. Examples will include ESI-MS analysis of opium, ESI-MS/MS analysis of methamphetamine and heroin bulk exhibits, ESI-MS/MS/MS analysis of cocaine samples, APCI-MS analysis of testosterones, APCI-MS/MS analysis of nandrolone decanoate, DESI-MS and DESI-MS/MS analyses of single and multi-component tablets.

**Screening, Controlled Substances, Mass Spectrometry**

## A163  The Stability of Cathinone in Dried Khat

*John S. Chappell, PhD*, and Marsha M. Lee, DEA Laboratory, 390 Main Street, Room 700, San Fransisco, CA 94105*

After attending this presentation, attendees will understand the long term stability of cathinone, the principle psychoactive component of the khat plant (Catha edulis), in the dried plant material.

This presentation will impact the forensic community by explaining how a quantitative study of dried khat samples has found cathinone concentrations to be relatively stable for a period of two years, and cathinone has remained at an identifiable level for over eight years. The study also determined simple drying techniques to be an effective means to preserve khat evidence for long term storage.

A primary concern with the forensic analysis of khat evidence has been the need to identify cathinone, which converts to other compounds, and predominantly to the less-active substance cathine in the harvested plant. The loss of cathinone has serious legal implications since cathinone is a schedule I controlled substance under federal regulations in the United States, while cathine is schedule IV. The propensity of cathinone to convert into cathine is regarded as the primary reason that cathinone was not isolated and identified from khat until the 1970's, following nearly one hundred years of chemical investigation. Early quantitative studies on the alkaloid content of khat found that the biosynthetic pathway for the plant is to produce cathinone in the rapidly growing plant tissues, and to reduce the cathinone to cathine via an enzymatic process in the more matured regions of the plant. The young shoots and tender leaves are consequently prized by khat consumers as they are the most actively growing portion of the plant and contain the highest concentration of cathinone. The conversion to cathine occurs in the young shoots after harvesting, and was once believed to occur rapidly upon drying and to continue with storage. However, the loss of cathinone upon drying is a matter of degree and is not necessarily complete. The drying of plant material by air or freeze-drying techniques has been frequently used in the research of khat to preserve the cathinone content

in the short term for future analyses. Further, air-dried and freeze-dried samples of khat have been found to contain consistent amounts of cathinone after several months in storage at room temperature, indicating that longer time periods of preservation are possible. Unfortunately, the misconception persists for some in the forensic community that cathinone is undetectable in dried or fresh khat after 48 hours of harvesting.

The current study employed high-performance liquid chromatography (HPLC) to quantitate cathinone, as well as the khat alkaloids cathine ((+)-norpseudoephedrine) and (-)-norephedrine. Two khat samples that were seized as dried plant material in 1999 were initially examined. Khat encountered in this dried form has been called "graba" in the United States. The cathinone concentration exhibited a measurable decrease over a two-year period, although the rate of decline was minor and has allowed cathinone to be readily detected for over eight years while stored at room temperature. The cathine and norephedrine levels remained essentially the same over this time period, suggesting that a reduction reaction is not responsible for the slow loss of cathinone. Additional study considered the drying of fresh plant material at room temperature and by the application of heat with either convection or microwave ovens. The dried preparations exhibited similar cathinone stability as the seized dried materials. The cathinone concentration in the heated materials were lower (approximately 30%) than in the air-dried preparation, but remained at a detectable level.

A forensic laboratory should make an attempt to preserve drug evidence such that reanalysis by another analyst or laboratory can confirm the same findings at a later time. While a crime laboratory may be able to make the identification of cathinone on relatively fresh samples of the khat plant, a concern has remained whether cathinone may be preserved in khat evidence for long term storage. The analytical findings of this study demonstrate that cathinone may persist in dried khat for a time frame of several years, and simple drying techniques and ordinary storage conditions may serve as an effective means of preserving seized khat evidence. The precise length of time that cathinone may remain detectable requires further study.

**Khat, Cathinone, Stability**

## A164  Available Without a Prescription - The Presence of Pharmaceuticals in Dietary Supplements

*Heather A. McCauley, BS*, Laura A. Ciolino, PhD, Angela S. Mohrhaus, BS, and Tracy L. Ranieri, BS, U.S. Food and Drug Administration, 6751 Steger Drive, Cincinnati, OH 45237*

After attending this presentation, attendees will have an overview of cases encountered by the FDA's Forensic Chemistry Center (FCC) involving dietary supplements and the methodology used to detect pharmaceuticals found in the products.

This presentation will impact the forensic community by providing an overview of pharmaceuticals being found in dietary supplements readily available to the public.

A dietary supplement, as defined by the Food and Drug Administration, is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet. The dietary ingredients in these products may include: vitamins, minerals, herbs or other botanicals. The Dietary Supplement Health and Education Act of 1994 (DSHEA) places dietary supplements in a special category under the general umbrella of foods, however it regulates supplements under a different set

of regulations than conventional food. Under these regulations, dietary supplement manufacturers are responsible for ensuring the safety of their product before it is marketed. The FDA is responsible for taking action against any unsafe dietary supplement only after it reaches the market. This, combined with the fact that manufacturers are generally not required to register their products with the FDA and the vast availability of products on the internet, provides an open invitation for many dietary supplement formulations to contain unlawful ingredients.

The Forensic Chemistry Center (FCC) has a history of analyzing a wide variety of nutritional supplements with weight loss supplements receiving more attention recently. The Chinese Journal of Public Health published a 2002 inspection of herbal weight-reducing dietary supplements on the market in a province in China which stated that approximately one-third of "natural" dietary supplements were found to contain prohibited drugs. Products for weight-loss commonly encountered by the FCC have contained compounds such as ephedrine alkaloids, 2,4-dinitrophenol, synephrine, and phentermine. According to a recent inspection and investigation, the adulteration of supplements marketed to and exported from China showed that the principal illegal adulterants were fenfluramine, phenolphthalein, sibutramine, and orlistat. Press Releases urging members of the public to not consume or buy certain weight-loss products containing these types of compounds have been emerging from areas such as Hong Kong and Canada for the past several years. In addition, analogs of these compounds are becoming more prevalent in these products. Some believe that manufacturers are adulterating their products with drug analogs to avoid being detected by ordinary laboratory methods. Regardless of the intention behind using drug analogs in dietary supplements, or more specifically in weight-loss products, the presence of these compounds is increasing and the occurrence of these products domestically continues to rise. Over the last year, the FCC has seen products containing desmethylsibutramine, di-desmethylsibutramine, and the N-formamide derivative of didesmethylsibutramine.

A recent survey of dietary supplements indicated for weight loss was conducted at the FCC. The samples were prepared using a MeOH extraction and analyzed using GC-MS. To broaden the scope of compounds detected by GC, the MeOH extracts are also derivatized with bis(trimethylsilyl)triflouracetamide. In addition, the samples were prepared for quantitative analysis by HPLC-UV using a 0.1N HCl extraction. Most products analyzed using GC-MS were found to contain sibutramine and/or sibutramine analogs. Moreover, based on the quantitative results some of the pharmaceuticals were present at therapeutic levels. In addition, other unrelated drugs were observed at trace levels in the GC-MS screen. It is suspected that poor manufacturing practices may have resulted in cross contamination adding to the problems observed with some of these products.

**Dietary Supplements, Prescription Drugs, Sibutramine**


# A165   MDMA Synthesis Affecting Canine Detection

*Michael S. Macias, BS, BS*, and Kenneth G. Furton, PhD, International Forensic Research Institute, Florida International University, University Park, Miami, FL 33199*

The goal of this presentation is to compare samples of 3,4-methylenedioxy-N-methylamphetamine (MDMA) pills to determine what differences exist in the chemical make - up and headspace odor, and if the differences affect detectability by trained law enforcement detector canine teams.

This presentation will impact the forensic community by demonstrating how seemingly unrelated MDMA samples possess similarities that are detectable by biologic and instrumental means.

The use of the illicit drug MDMA (also referred to as Ecstasy and X) has increased in recent years among adolescents and late-night partiers (i.e., "Ravers"). MDMA was first developed in Germany in 1914 as a precursor for other drugs. Abuse in the United States is believed to have begun sometime in the 1960's on the west coast, and while it is traditionally taken in pill form, the drug is also available in powder and liquid form. There are a plethora of published processes for the chemical synthesis for MDMA most of which begin with a methylenedioxy compound. The most common of these compounds include safrole, isosafrole or piperonal, all of which are commercially available. It is widely agreed that MDMA causes a reduction in serotonin levels, but there is not agreement as to the severity of the effect. As a result of increased interest and usage, the distribution of this drug has increased in metropolitan and suburban areas across the country. MDMA is one of the top controlled substances most identified in crime labs, and it is the most recent drug to be added to law enforcement detection canine training regimens.

Despite the increasing number of instrumental methods for detection of characteristic chemical odors, the use of trained canines as biological detectors remains one of the most widely accepted methods to reliably detect drugs. Therefore, detector-dog response is one of the major applications involved with odor detection studies; both for the determination of the chemical signature of individual odors to which these canines are actually alerting, and to whether or not there is a common element within different items to support the use of contraband mimics. Previous studies have shown that law enforcement detection canines which are trained on real, representative samples containing actual parent compounds of drugs and explosives can and will alert to mimics based upon the dominant volatile odor compounds (VOC) found in the headspace of the parent compounds. Previously, piperonal has been shown to be one of the dominant odor compounds in the headspace of samples of MDMA, as well as a key odor to which MDMA trained law enforcement detector canines will alert.

Chemical analysis of MDMA solutions were performed using GC-MS and LC-MS/MS in both negative and positive modes, allowing for a full spectrum of detection. Solutions were prepared by grinding MDMA tablets and dissolving them in solvent. Headspace analysis was performed using SPME-GC-MS to identify the dominant odor compounds that are present in the samples. Upon analysis, a direct comparison was made to show how several compounds [such as 3,4-methylenedioxyphenylacetone and 1-(3,4-methylenedioxyphenyl)-2-propanol] are common among many samples of MDMA regardless of starting compound or synthesis procedure. In addition, differences that were found, such as levels of the various methylenedioxy starting compounds, can be shown to affect the overall outcome of detection alluding to the need of additional training aid odor identification and development.

**MDMA, Canine Detection, SPME**

## A166 Statistical Drug Sampling I: Training Narcotics Custodians in the Sampling of Large Marijuana Seizures

*Elizabeth A. Gardner, PhD\*, University of Alabama at Birmingham, Department of Justice Sciences, UBOB 210, 1530 3rd Avenue South, Birmingham, AL 35294-4562*

After attending this presentation, attendees will be able to understand how to calculate the number of random samples that must be selected from a population in order to be 95% confidence that 90% of the items in a population are a controlled substance. The attendee will also have the tools to generate their own table of the Hypergeometric distribution for a range of populations.

This presentation will impact the forensic community by presenting the Hypergeometric Equation in an easily digestible form and to serve as a model for the training of non - scientific personnel in the statistical sampling of large drug seizures.

Backlog is one of the most important issues in forensic crime labs. A training program was developed for narcotics custodians in the sampling of large marijuana cases in order to reduce the backlog of suspected controlled substances waiting for analysis. The objective of this presentation is to use simple statistics to derive the equation:

$$P_0 = [N_1!(N\text{-}n)!]/[(N_1\text{-}n)!(N)!] \le \theta \qquad \text{Eq. 1}$$

This equation is equivalent to the Hypergeometric Equation, assuming zero negative samples. After attending this presentation, attendees will be able to understand how to use Eq. 1 to calculate the number of random samples that must be selected from a population in order to be 95% confidence that 90% of the items in a population are a controlled substance. The attendee will also have the tools to generate their own table of the Hypergeometric distribution for a range of populations.

Teasing out the meaning of the equations used in statistical sampling can be one of the most difficult aspects of developing protocols for drug analysis. The impact of this work on the forensic science community is by presenting the Hypergeometric Equation in an easily digestible form and to serve as a model for the training of non-scientific personnel in the statistical sampling of large drug seizures.

There are about as many ways to sample populations in a drug seizure as there are labs that perform the analysis of controlled substances. Some methods are straightforward, as in testing for the presence of a controlled substance to prove possession or in testing to the lower limit of a penalty range. There are also 'accepted methods' arbitrary methods that include everything from *testing one*, *testing the square root of a population*, all the way to *testing all*.

However, the disadvantage of non-statistical methods is that the results of the tests performed on the samples do not reflect the composition of the entire population. For this, a statistical sampling method is required. There are several statistical methods that have been recommended for use by drug analysts. These include the Hypergeometric Distribution, The Binomial Distribution, and the Bayesian EquationApproach. The most commonly applied method is the Hypergeometric method. The strength of the Hypergeometric Equation is that it can be used to calculate the number of random samples to be selected even in the case where 1 or 2 negatives might be encountered during the testing. In reality though, most labs determine the number of samples that need to be analyzed by assuming that zero negatives will be encountered and then reading the number of random samples that must be selected from a table where the Hypergeometric Distribution has already

been calculated. The goal of this presentation is to demonstrate how to solve the Hypergeotric Equation and how the tables are generated.

There are three concepts that are essential for deriving Eq. 1. The first concept is simple probability:

$$P(E) = n(E)/n(S) \qquad \text{Eq. 2}$$

Where P(E) is the probability for E, n(E) is the number of desired outcomes and n(S) is the total number of possible outcomes. The second concept is that of N factorial (N!):

$$N! = N \text{ x } (N\text{-}1) \text{ x } (N\text{-}2) \text{ x } (N\text{-}3)\ldots\text{ x } 1 \qquad \text{Eq. 3}$$

Where N is any integer. The final concept is N choose *n*:

$$(N\text{-}n)!/(N!n!) \qquad \text{Eq. 4}$$

This equation is used to calculate the number of ways that n items can be selected from a population of N items when order does not matter. These three concepts will be used to generate Eq. 1, which will is all that is required to build a table of the Hypergeometric Equation.

**Statistical Sampling, Hypergeometric Distribution, Illicit Drugs**

## A167 YHRD 3.0 – An Improved Version of the YSTR Haplotype Reference Database for the Calculation of Match Probabilities

*Sascha Willuweit, MSc\*, Charite Institute of Legal Medicine, Department of Forensic Genetics, Hannoverschestr. 6, Berlin, 10115, GERMANY*

The goals of this presentation are to access/query contribution by accession numbers, search the database with a given haplotype, interpret matches and frequency calculations, build/query a custom reference database, and perform an online AMOVA analysis

This presentation will impact the forensic community by demonstrating the calculation and interpretation of match probabilities, demonstrating further sampling of Y-chromosomal STRs and population genetics.

The successful implementation of Y-STR analysis in forensic practice led to the establishment of large web-based population databases which facilitate the assessment of match probabilities for haplotypic profiles. Thanks to international collaboration the current release 24 of the Y-STR Haplotype Reference Database (YHRD)[1] consists of nearly 59,000 haplotypes from 499 population samples. The database has been online for 8 years with regular updates to meet the requirements of a broad community of users. However, recent developments in the forensic field and a still growing number of users made it necessary to rework the database for its next major version: YHRD 3.0.[2]

New capabilities, new features to improve usability and the enhancements of the existing functional range will be presented. Major changes originate from the inclusion of all types of mutation and the expansion of the set of markers available through the new underlying database. This expansion includes both Y-STR Loci (e.g., full Y-Filer coverage) and Y-SNP haplogroups (see SNPY nomenclature[3]).

The implementation of an AMOVA module (pairwise $\Phi_{ST}$ values and MDS plot are calculated) enables the user to study genetic distances between population samples from the YHRD and those submitted by the user. A reprogrammed mapping module to visualize the geographic distribution, the possibility to submit batch queries and a new export functionality, make the database an even more powerful scientific tool.

Another improvement is a module called "Custom Search", which enables the user to search in an own reference set of population samples. This is done by creating a new "Custom Database" by picking appropriate populations from the YHRD database set. There are pre-defined

databases (e.g., countries) which enable the forensic user to search the YHRD like a national reference database.

**References:**
[1]  Willuweit S, Roewer L, on behalf of the International Forensic Y Chromosome User Group (2007) Y chromosome haplotype reference database (YHRD): Update, Forensic Science International: Genetics 1(2) 83-87
[2]  http://www.yhrd.org
[3]  http://www.snp-y.org

**Y-Chromosomal STRs, Forensic Reference Database, Population Genetics**

## A168 The Analysis of Defined Data Sets of Mixture STR Profiles Using Several Mixture Deconvolution Tools

*Rhonda K. Roby, PhD, MPH\*, NEST Project, 3500 Camp Bowie Boulevard, Room 310, Fort Worth, TX 76107; Valerie K. Bostwick, MS, Eugene N. Brooks, BS, Sally Edwards, BS, and Terry W. Fenger, PhD, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701; and John Paul Jones, MBA, National Institute of Justice, Office of Science and Technology, 810 7th Street, Northwest, Washington, DC 20531*

The goal of this presentation is to take a defined data set of simulated STR mixtures and process the data through several mixture deconvolution tools available to the forensic community.

This presentation will impact the forensic community by providing a survey of additional tools available to the forensic scientist for the evaluation of mixed STR DNA profiles.

Mixture deconvolution tools, also known as fancy calculators, have been designed by several programmers/companies to assist forensic scientists in mixture interpretation of casework STR data. Mixture results pose an additional challenge in case interpretation and can be quite time-consuming, even for the experienced forensic scientist. Cases involving sexual assault, homicide, and touch DNA often have a mixture of two or more DNA profiles. As advances are being made in the forensic community with expert systems for single source DNA interpretation, more and more focus is being directed at other software tools that can assist the forensic examiner in interpretation of mixed STR profiles.

Controlled mixture studies were conducted to produce two data sets; each data set used a different pair of male and female DNA samples. The design of the mixture samples included varying ratios of the male and female DNA at 30:1, 10:1, 3:1, 1:1, 1:3, 1:10, and 1:30 with various DNA input levels. The different amounts of DNA added to each amplification (i.e., 1.5X, 1.0X, 0.5X, and 0.25X) were based on the manufacturers' published recommendations. These varying ratios and varying input quantities of DNA were amplified with PowerPlex® 16 System (Promega Corporation, Madison, Wisconsin) and AmpfℓSTR Identifiler®, Profiler Plus®, COfiler®, and SGM Plus® PCR Amplification Kits (Applied Biosystems, Foster City, California). All samples were run on multi-capillary electrophoresis instruments.

The raw data were analyzed using several mixture deconvolution tools and calculator packages. These include but are not limited to: DNA_DataAnalysis Software (United States Army Criminal Investigative Laboratory, Fort Gillem, Georgia); FSS-i3™ Expert Systems Software version 4.1.3 (Promega Corporation) in conjunction with GeneMapper® *ID* Software version 3.2 (Applied Biosystems);

GeneMapper® *ID-X* Software (Applied Biosystems); and, TrueAllele® Casework System Package (Cybergenetics, Pittsburgh, Pennsylvania). The results of these studies demonstrate that fancy calculators can positively identify a partial profile of a minor contributor even at low ratios amplified with 0.25 ng total DNA. All of these tools can evaluate two-person DNA mixtures and produce best-fit major profiles and others can provide invaluable assistance with three-person mixed profiles. Each of the mixture deconvolution tools interprets the mixed data using mathematical modeling and algorithms from output peak definition and peak height information for each of the amplifications. Some of the software packages report weighted ratios whereas others report proportions. It is clear from this evaluation that the different mixture deconvolution tools address stutter differently and ask very different questions of the data. For example, the knowledge base of one software program uses no *a priori* information regarding the mixtures, whereas another software program allows the user to define one reference, for example the victim, in its interpretation. One critical observation is that the software packages perform the calculations in the same manner every time producing unbiased and reproducible results. These comparisons and the results from the different software packages will be discussed.

The focus of this presentation is to share information about the different mixture deconvolution tools that are available to the forensic community, both commercially and as freeware. Through surveying the different mixture deconvolution tools, it is clear that the knowledge base of each software program is different and that they are each querying different parameters. It is the intent of this presentation to share the advances made with each software program and their respective limitations.

**Mixture Profiles, Deconvolution Tools, Software**

## A169 Development of an Automated Approach for the Deconvolution of DNA Mixtures

*Lisa M. Calandro, MPH\*, Nicola Oldroyd, BS, Ravi Gupta, MS, Thomas McElroy, BS, Bruce Desimas, MS, and Xi Hau, MBA, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will have learned a new automated mixture analysis tool that helps to alleviate a significant bottleneck in the DNA analysis process thereby increasing overall DNA laboratory throughput.

This presentation will impact the forensic community by increasing awareness of an automated mixture analysis and statistical calculation tool which has the potential to decrease interpretation time and improve the time to result for DNA cases.

Crime laboratories are increasingly adopting automated systems as a means of increasing sample processing throughput. As a result of successful implementation of these high-throughput systems, laboratories are faced with the task of reviewing a growing amount of sample data, effectively moving the bottleneck downstream. Casework analysts spend the majority of their hands-on time in two areas, a) the screening and documentation of items containing biological evidence and b) the analysis of complex electronic data to maximize information recovery

from DNA samples, especially those containing mixtures. The interpretation of DNA mixtures presents particular challenges for the casework analyst. Significant variation exists in the procedures for evaluating DNA mixtures between laboratories, as they are not standardized, resulting in potentially different interpretations of the same data. In addition, analysis is complicated by factors inherent to fragment analysis which may impact a mixed DNA profile such as: allele dropout due to low input amounts of sample DNA, stutter, artifacts, and heterozygote peak balance at various input amounts.

The automated mixture analysis tool is designed to streamline the interpretation of profiles from mixed DNA samples by automating functions which would otherwise be performed manually. The mixture analysis tool assists the casework analyst in performing:

a) Segregation of samples based on the minimum number of contributors
b) Deconvolution of 2-person mixtures into contributor genotypes
c) Statistical analysis of samples incorporating a variety of approaches

An approach to the deconvolution of 2-person mixtures into individual profiles, also referred to as the major and minor contributor, will be described. The approach leverages two key inferences: (1) At any locus, two alleles originating from the same person have roughly the same height, and (2) Established mixture proportions remain consistent across all loci within a sample profile. The approach results in a set of genotype combinations that are scored based on consistency of the mixture proportion at all loci across the profile and heterozygote peak height ratio (measured against user-defined thresholds). An "Inclusion Quality" Process Component-based Quality Value (PQV) is generated which provides an assessment and ranking of the set of genotype combinations. The genotype combinations are segregated into 2 tables based on passing or non-passing Inclusion Quality values. All genotype combinations are available for review by the analyst within the sample plot, allowing analysts to verify the mixture interpretation and make changes based on their knowledge and experience. The software then utilizes the resulting profiles to compute Random Match Probability (RMP), Combined Probability of Inclusion/Exclusion (CPI/CPE) and Likelihood Ratio (LR) statistics commonly utilized by forensic practitioners to provide a measure of the weight of the evidence. All information generated by the tool can be exported for record keeping.

**Data Analysis, Mixture Analysis, DNA**

## A170   Fighting Human Trafficking With DNA — The Prokids Program

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After attending this presentation, attendees will be informed about the problems behind human trafficking and how DNA databases can help to solve and prevent this terrible crime.

This presentation will impact the forensic community by showing new applications of DNA analysis and by showing how international tutoring programs and collaboration will help in this case.

According to U.N. reports and according to different Government and Non-government agencies, human trafficking is becoming one of the main criminal problems and it is slowly becoming the most important crime in economical terms. There are different ways to fight this crime, but one of them has a scientific, criminalistic approach.

It is known that missing persons identification is a collaborative effort where DNA analysis is usually of the greatest help, specially in those cases where samples are degraded or the identification is based in badly preserved bones or partial skeletons.

In 1997, Spain established one of the first "Missing persons DNA identification" programs, developed by the Dept. of Legal & Forensic Medicine of the University of Granada and the Guardia Civil (the largest Spanish national law enforcement agency). A lot of experience was gained, and as of July 2008, over 190 remains were identified using DNA as the primary tool; once a DNA match is found, other forensic sciences are applied to confirm the identification, such as anthropology or odontology.

Nevertheless, DNA analysis is also useful to identify "other kind" of missing persons that are not dead, as it is the case of missing children. It is estimated that over 1 million kids are actively reported as "missing", although there is no doubt that there are many more missing and not reported by different reasons. Most of these kids have been taken apart from their families and are being exploited or trafficked.

At the University of Granada and with the support of the BBVA, F. Marcelino Botin and the GENNA Foundation, we have developed the PROKIDS Program to generate two independent databases that are automatically compared each other every time a new profile is entered. The first database or Reference Database (KD) is composed of DNA profiles (autosomal, Y chromosome, and mitochondrial data, basically) obtained from voluntarily donated biological samples (buccal swabs) from mothers and other family members of missing kids. Efforts are focused in obtaining samples from the biological mother in all cases, since –according to different statistics- in some areas were illegal adoptions are common, up to 25% of the alleged fathers are not the biological ones, and this fact could compromise the effectiveness of the database.

The second database or Questioned Database (QD) is composed of DNA profiles (autosomal, Y chromosome, and mitochondrial data, basically) obtained from kids that have been found without their families, or that are being exploited or known as victims of human trafficking. In 2009, autosomal and chromosome-Y SNPs will be introduced as a new tool with to different but parallel objectives; first, to obtain more statistic power in the final calculations and conclusions, and second, to be able to obtain information that could give an investigative lead regarding the geographical origin of a missing kid.

The University of Granada has already signed agreements with the governments of Mexico and Guatemala (other countries are ready to sign now) to help to collect and analyze – if necessary – the samples, and in any case, to coordinate the databases. So far, over 250 samples from missing kids have been introduced in the QD, and 354 biological samples of relatives are in the RD.

There is no doubt that a global, coordinated effort is necessary to help to stop and prevent this terrible crime, and forensic science, with the use of DNA analysis, can play an important, leading role in this attempt.

**DNA, Genetic Databases, Human Trafficking**

## A171   A Match Likelihood Ratio for DNA Comparison

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After attending this presentation, attendees will understand how a general approach to infer genotypes with uncertainty can be used, and then match them using a generic match likelihood ratio (MLR) statistic.

This presentation will impact the forensic community by introducing and motivating the "match likelihood ratio." This presentation gives intuitive examples that show how MLR already appears in current DNA analysis, and discuss the inherent limitations of some earlier classical LR methods. This presentation will show how MLR transcends these limitations, and handles modern DNA problems, such as missing persons, familial search, low copy number, mass disasters, touch DNA, and complex mixture interpretation. The use of MLR may help forensic scientists extract more match information from their current DNA data. Moreover, it may help translate protocols for complex DNA data into admissible statistical evidence.

Forensic scientists analyze DNA evidence to determine a strength of association between two biological specimens. When feasible, they use a likelihood ratio (LR) to describe this association, since the LR removes all considerations except of the weight of DNA evidence. The LR helps the police in DNA investigation and the prosecutor with presenting DNA evidence. The classical likelihood ratio is used routinely for identifying individuals (random match probability), kinship (paternity index) and mixtures (combined likelihood ratio).

Recent years have seen the advent of other, equally important, forensic DNA applications. These applications include missing persons, familial search, low copy number, mass disasters, touch DNA and complex mixture interpretation. Each of these approaches entails the analysis of uncertain DNA data, which may include more than one genotype possibility. However, there is no generally accepted statistical approach that can determine the match rarity for all of these applications.

The authors have recently developed a general approach to constructing a match likelihood ratio (MLR) for all such DNA comparison problems.[1] The forensic scientist first infers a genetic profile, representing genotype uncertainty using probability. Then, genetic profiles are compared using a straightforward sum of products formula that computes the match likelihood ratio.

For example, the standard Combined Probability of Inclusion (CPI) mixture statistic can be shown to be a match likelihood ratio. Every allele pair that is included in the allele data becomes a genotype possibility; each such possibility is then assigned an equal genotype probability. The CPI statistic is obtained by substituting these inclusion genotype probabilities into the MLR formula. This approach establishes that CPI is indeed a likelihood ratio, and also illustrates how genotype probabilities are regularly used in current forensic DNA practice.

The MLR inference approach is based on Bayesian reasoning. Genotype inference is shown to result from the combination of a likelihood function for assessing data, and a prior probability. Uncertainty in genotype values is represented using probability. Any such inferred genotypes can be substituted into the general MLR formula in order to obtain a rigorous likelihood ratio statistic that describe the rarity of genotype match.

Inferring genotypes, and then comparing them in a general match likelihood ratio, can be helpful in: (1) performing objective, unbiased comparisons, (2) using highly informative genotypes inferred from statistical or "expert system" computing, (3) separating crime scene and suspect DNA workflow processes, (4) enabling sophisticated computer matching on genotype databases, (5) providing a general framework for current forensic match approaches, (6) quantifying the strength of case-to-case matches to link crime scenes, and (7) validating and comparing different DNA interpretation methods for efficacy and reproducibility.

This presentation introduces and motivates MLR. It gives intuitive examples that show how match LR already appears in current DNA analysis, and discuss the inherent limitations of some earlier classical LR methods. It shows how MLR transcends these limitations, and handles modern DNA problems, such as missing persons, familial search, low copy number, mass disasters, touch DNA and complex mixture interpretation. The use of MLR may help forensic scientists extract more match information from their current DNA data.

**Reference:**

1   Perlin MW, Kadane JB, Cotton RW. Forensic DNA Inference. In: Seventh International Conference on Forensic Inference and Statistics; 2008 August; Lausanne, Switzerland.

**Forensic DNA, Likelihood Ratio, Familial Search**

## A172   Identifying Victim Remains From Uncertain Data

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After attending this presentation, attendees will understand how they can analyze uncertain mass disaster DNA data (victim remains, personal effects, family references) by inferring genotypes (up to probability) and matching them with a likelihood ratio statistic.

This presentation will impact the forensic community by describing how computer tools can help forensic analysts overcome mass disaster data uncertainty, and thereby make reliable identifications, in the context of the World Trade Center (WTC) STR data. A mass disaster can generate a vast amount of biological material. Such materials include the victim remains evidence at the disaster site, as well as the personal effects and family references of the missing people. These data may have considerable uncertainty, which occurs at many levels. Current reference sample expert systems do not address genotype uncertainty, so instead newer statistical computing methods are needed.

A mass disaster can generate a vast amount of biological material. Such materials include the victim remains (VR) evidence at the disaster site, as well as the personal effects (PE) and family references (FR) of the missing people. These data may have considerable uncertainty, which occurs at many levels.[1] Current reference sample expert systems do not address genotype uncertainty, so instead newer statistical computing methods are needed. This paper describes how computer tools[2] can help forensic analysts overcome mass disaster data uncertainty, and thereby make reliable identifications, in the context of the World Trade Center (WTC) STR data.

At the DNA level, VR samples are often degraded, mixed, burned, contain minimal DNA, or are compromised in other ways. PE samples are collected from contaminated environments, and may include mixed or

degraded DNA. With FR samples, there can be uncertainty in the family kinship relationships. Human visual review of the STR data is needed to determine which data are good, mixed or unusable.

Indeed, the STR lanes (or injections) associated with a particular sample may be derived from different individuals. To disambiguate the data, we use a visual user interface software tool that lets an analyst view all of the provided lanes for a given sample, and then group compatible lanes together to form one or more genetic calculation requests for computer interpretation of that sample. In the WTC workflow, a visual user interface operator can inspect data and generate interpretation requests for 30 VR samples every hour.

When inferring a profile from uncertain mass disaster data, the resulting DNA profile may include more than one genotype. This profile uncertainty can be represented by assigning probabilities to genotypes.[3] The forensic analyst can use a commercially available genetic calculator[2] to infer genotypes in many common scenarios. For example, the calculator can infer profiles from mixtures having two or more unknown contributors, statistically combine uncertain data from multiple lanes, and infer a missing person's profile from kinship data.

To compare victim remains profiles with missing person profiles, the calculator's DNA match module[2] computes a likelihood ratio that compares the probability of a victim remains match with a missing person to that with a random person. Every match comparison in the system, whether kinship or STR, has a numeric likelihood ratio score that measures the degree of DNA identity.[3]

To help the forensic analyst integrate all of this profile and match information, the visual user interface provides visual representations at each locus of the genotype contributor probabilities and match likelihood ratios. The original EPG data can be seen and explored by the user at all times. A match-directed sample review takes only a few minutes, since the user can interactively focus on interesting questions about data, genotypes or matches.

A subset of the WTC data for 18,251 VRs and 2,386 PEs have been visually reviewed and statistically reanalyzed. Additionally 2,347 missing person profiles have been genetically reconstructed from 6,660 FRs. In a mass disaster project of this magnitude, forensic analyst expertise is essential for examining the data to ask the orrect questions, and assess the calculated answers.[1] Computer tools, including a visual user interface and a 24 processor genetic calculator supercomputer[2] for statistical genotype inference and matching,[3] can perform genetic calculations that help analysts apply their expertise to obtain reliable identifications with less effort.

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1  Perlin, MW. "Mass casualty identification through DNA analysis: overview, problems and pitfalls." Forensic Investigation and Management of Mass Disasters. MI Okoye and CH Wecht. Tucson, AZ, Lawyers & Judges Publishing Co: 23-30, 2007.

2  Perlin, MW. "Identifying human remains using TrueAllele® technology." Forensic Investigation and Management of Mass Disasters. MI Okoye and CH Wecht. Tucson, AZ, Lawyers & Judges Publishing Co: 31-38, 2007.

3  Perlin MW, Kadane JB, Cotton RW. Forensic DNA Inference (Abstract). In: Seventh International Conference on Forensic Inference and Statistics; 2008 August; Lausanne, Switzerland.

**Forensic DNA, Mass Disaster, Computer Software**

## A173  Enhanced Quantitation Data Analysis Using Next Generation Real - Time PCR Analysis Software

*Alan B. Dixon, MSFS\*, Jacki Benfield, BS, Lisa M. Calandro, MPH, Heidi L. Kijenski, BS, Thomas McElroy, BS, Maura Barbisin, PhD, Andrew Masel, PhD, Makesh Karpagavinayagam, MS, Gloria Lam, MBA, Chris Vinson, BSIS, and Allan Minn, BS, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will learn about the next generation real-time PCR analysis software which streamlines quantitation set-up and data analysis as well as provides sample dilution and STR reaction set-up tools.

This presentation will impact the forensic community by explaining how the next generation real-time PCR analysis software will provide efficient analysis of quantitation data, assist in obtaining optimal results from downstream STR reactions, and enable continued high quality laboratory throughput.

As forensic laboratories continue to seek ways to increase throughput, the need for advanced software has become paramount. The use of real-time PCR systems for DNA quantification and quality evaluation has reduced analysis time and provided highly informative results. The ability to quantify the DNA from large amounts of samples requires software that can aid the forensic scientist in the critical analysis of data from a wide range of sample types. The next generation real-time PCR analysis software has been developed to meet this need by establishing a Quality Control Flag system to ease data analysis by quickly and accurately identifying sample or quantitation assay anomalies. Furthermore, simplified background, ROI, optical, and pure dye spectral calibrations complete with wizard-based instructions and automated analysis have been incorporated into the next generation real-time PCR analysis software.

The next generation real-time PCR analysis software employs a Quality Control Flag system to assist the analyst with streamlined data analysis and evaluation of critical information obtained from quantitation assays. Such information includes the detection of PCR inhibition, reagent contamination, and mixtures of male and female DNA. Quality flags evaluate the slope, $R^2$, and Y-intercept standard curve metrics, Internal PCR Control (IPC) $C_T$ value, high or low quantity samples, and instrument performance. In addition, a Male to Female ratio flag specific to the multiplexed quantitation assay indicates the presence of samples containing a mixture of male DNA combined with excess female DNA prior to STR analysis.

Each of these quality flags not only eases data analysis but provides guidance for downstream STR analysis. By automatically assessing sample quality and quantity, the software helps to facilitate the selection of the appropriate STR amplification kit and DNA input amount, or whether further processing is required. For example, when the IPC $C_T$ flag is activated, the analyst can evaluate sample specific amplification plots to determine if dilution and requantification is required or if the sample should be amplified using a mini-STR kit due to sample inhibition. Also, if the user defined Male to Female ratio flag is triggered in the multiplexed quantitation assay, the analyst can determine if autosomal or Y chromosome amplification should be performed. The software also contains workflow enhancements to assist the analyst in preparing sample dilutions for STR reactions of choice as well as provide STR reaction set-up parameters. The next generation real-time PCR analysis software has been designed to provide efficient analysis of

quantitation data, assist in obtaining optimal results from downstream STR reactions, and enable continued high quality laboratory throughput.

**Next Generation Real-Time PCR Analysis Software, Quality Control Flags, Multiplexed Quantitation**

## A174 Investigations on the Use of Samplematrix to Capture and Stabilize Crime Scene Samples

*Katherine A. Roberts, PhD*, and Donald J. Johnson, MS, California State Los Angeles, School of Criminalistics, 5151 State University Drive, Los Angeles, CA 90032*

After attending this presentation, attendees will have a better understanding of the stabilization properties of samplematrix.

This presentation will impact the forensic community by providing data on alternate ways of capturing and storing biological fluids.

The successful resolution of crime scene investigations often depends on the ability to identify and individualize biological evidence. Fundamental to this analysis is the stabilization of biological evidence, because testing generally does not proceed immediately after collection. Crime scene samples and liquid extract derivatives are routinely stored frozen in forensic laboratories, to be thawed at the time of analysis. However, numerous lines of evidence indicate that the process of freezing and thawing has detrimental effects on biological samples. Many crime scene samples are sub-optimal for analysis—the further degradation of the samples by current storage systems only exasperates the difficulty of identifying and individualizing biological evidence.

Biomatrica, Inc. has developed a proprietary platform technology for the dry storage of biological materials at ambient temperatures. The key component of this technology is SampleMatrix™, which was derived from studies on extremophile organisms. These organisms can survive extreme environmental conditions. SampleMatrix™ consists of protective agents developed from combining small molecule chemistry with advanced polymer chemistry. This product has multiple components: (1) the dissolvable polymer in a stabilization buffer adjusted for the different biological samples, and (2) a stabilizing solution containing small synthetic molecules.

Research on the effectiveness of SampleMatrix™ to stabilize biological evidence as compared to conventional storage methods has been conducted. The study tests the hypothesis that the storage of biological samples, swabs and liquid DNA extracts, in the SampleMatrix™ polymer at room temperature reduces the exogenous degradation of DNA by minimizing the adverse effects of hydrolysis and freeze-thawing. Blood, semen, and saliva stains of different concentrations were initially deposited on five different substrates. Each stain was sampled by swabbing with water or SampleMatrix™ as the wetting agent and then exposed to room temperature v. freezer storage for longitudinal study. In addition, we have conducted parallel studies whereby blood, semen, and saliva (of comparable concentrations to the substrate study described above) were directly applied to swabs and subsequently stored for longitudinal study.

This presentation will discuss the results of the experimental conditions that were evaluated by Real-Time and STR analysis following standard forensic protocols

**Stabilization, Crime Scene Collection, Biological Stains**

## A175 Towards the Development of a Single Microfluidic Device for DNA Extraction using Magnetic Particles and Non-Contact IR-Mediated PCR for STR Analysis

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After attending this presentation, attendees will have learned about the development of a microfluidic device that combines solid phase extraction using magnetic particles with infrared - mediated PCR amplification of DNA for STR analysis.

This presentation will impact the forensic community by presenting work that represents a major step towards the development of a fully integrated microdevice capable of total DNA analysis for forensic casework.

The proven utility of forensic DNA evidence has increased the demand for DNA analysis services. Although conventional analysis techniques are effective, they are time consuming and laborious, which has contributed to an overwhelming backlog of forensic casework samples with possible biological evidence. Microchip technology offers the potential of a rapid, cost-effective alternative to conventional DNA analysis methods. Microdevices provide self-contained, closed systems for analysis procedures, diminishing the potential for contamination or loss of sample. In addition, the use of microchips offers a reduction in required sample volume, which could potentially allow for the analysis of casework not amenable to conventional procedures. Techniques performed on microchips are particularly advantageous because they can be integrated with upstream or downstream analytical steps on a single microfluidic device in the form of a lab-on-a-chip. These integrated microfluidic systems, which incorporate all of the sample processing steps required for DNA analysis, will reduce analysis times, and therefore, the forensic casework backlog.

Successful integration of microchip packed bed solid phase extraction (SPE) and infrared-mediated (IR-mediated) PCR – two of the procedures necessary for forensic genetic analysis of crude biological samples – has previously been demonstrated by our laboratory.[1, 2] Recently, an alternative microchip SPE method (ωSPE) has been developed that utilizes commercially available paramagnetic particles. An external magnet is used to control the location of the particles in a microfabricated chamber, removing the need for etching structures (such as weirs or pillars) into the channels, and increasing the simplicity of device design and fabrication. The ωSPE technique provides similar or better extraction efficiencies and increased capacity compared to packed bed microchip SPE techniques. In addition, ωSPE allows for user-defined fluidic control, eliminating carryover from incompatible SPE reagents (chaotropic salt or organic wash solvents) that would inhibit subsequent PCR amplification – a particular challenge in the integration of microchip SPE and PCR.

The research presented will highlight the development of integrated microdevices that combine solid phase extraction using magnetic particles (ωSPE) with infrared-mediated PCR (IR-PCR) amplification of the purified DNA. The functionalities of the device are described, including the results of separations of the STR fragments from genomic DNA isolated and amplified using the integrated device. The presented

work represents a major step towards the development of a fully integrated microdevice capable of total DNA analysis for forensic casework.

**References:**

1. Legendre, L.; Bienvenue, J.; Roper, M.; Ferrance, J.; Landers, J., A valveless microfluidic sample preparation device for DNA extraction and amplification using conventional instrumentation. *Anal Chem* **2006,** 78, 1444-51.
2. Bienvenue, J.; Legendre, L.; Ferrance, J.; Landers, J. In *Integrated DNA extraction and PCR amplification of STRs: interfacing microfluidic devices with current methodologies and conventional instrumentation*, American Academy of Forensic Sciences 59th Annual Scientific Meeting, San Antonio, TX, 2007; San Antonio, TX, 2007.

**DNA Extraction, PCR, Microchip**

## A176   DNA Purification From Forensic Samples in a Microfluidic Biochip

*Eugene Tan, PhD*, Network Biosystems, 1B Gill Street, Woburn, MA 01801*

After attending this presentation, attendees will be familiar with recent advances in biochip-based DNA extraction and purification protocols that enable forensic sample preparation to be performed rapidly and with minimal user intervention.

This presentation will impact the forensic community by demonstrating biochip-based DNA extraction and purification, a major step towards the development of a fully integrated, samples-in to results-out STR analysis system for both laboratory use and field forward operation. Such a system has the potential to reduce the time, labor, and cost of performing STR analysis.

A major challenge in bringing biochip-based DNA analysis tools to the forensic community has been in developing a robust, easy to operate commercial instrument that offers reliable and reproducible performance. A fully integrated sample-in to results-out biochip-based DNA analysis system specifically for human identification will be discussed. This system comprises three microfluidic modules to perform: (1) DNA extraction, purification, and human specific quantification, (2) multiplexed STR amplification, and (3) separation and detection of the resulting amplicons. In developing such a system, it is critical that each module function at least as well as—and preferably better than—the analogous conventional technology that it is designed to replace. We have previously reported on Genebench-FX™ Series 100, a microfluidic biochip-based separation and detection system that is ruggedized for operation in the laboratory and field. The instrument allows multiplexed STR amplification products to be separated and detected with single basepair resolution, high precision, and high sensitivity in under 15 minutes.

We will report on the development of a module for rapid biochip-based DNA extraction and purification. Single use disposable microfluidic biochips capable of simultaneously purifying 8 or 16 samples were designed and fabricated. Fluidic manipulation within the biochips was accomplished with active and passive valves, pumps, and air pressure. A purification membrane incorporated within the biochip binds DNA from the lysate. Subsequent washing of the bound DNA removes contaminants and purifies the bound DNA. Purified DNA eluted from the membrane meets volumetric and quality requirements for subsequent biochip processing. The extraction and purification protocol is automated by instrumentation developed to apply pressure and vacuum to the input ports of the biochip in a sequential manner according to a computer-controlled script. Optimization of the reagents and protocol allows simultaneous purification of 8 or 16 samples in 10 minutes. Biochip-purified DNA is quantified conventionally and amplified by rapid biochip-based PCR, and amplification products are characterized by separation and detection on Genebench-FX™ Series 100.

Data will show that non-probative and mock casework samples including buccal swabs, dried blood, and whole blood samples are purified with high efficiency and that the resulting DNA is compatible with subsequent PCR amplification and separation and detection. It is demonstrated that biochip-based DNA purification is well-suited for incorporation into a fully-integrated microfluidic forensic DNA analysis system.

**STR Analysis, DNA Extraction and Purification, Biochip**

## A177   Rapid STR Amplification in Conventional and Biochip Systems

*Eugene Tan, PhD*, Network Biosystems, 1B Gill Street, Woburn, MA 01801*

After attending this presentation, attendees will become familiarized with recent advances in biochip based DNA analysis systems and, in particular, with a biochip-based rapid multiplex PCR module that enables STR amplification to be performed in approximately 17 minutes.

This presentation will impact the forensic community by demonstrating biochip-based multiplex amplification, a major step towards the development of a fully integrated, samples-in to results-out STR analysis system. The rationale for developing biochip-based DNA analysis tools is that a fully integrated system has the potential to reduce the time, labor, and cost of performing STR analysis. These advances may increase the capacity of forensic laboratories as well as reduce the current backlog of casework and database samples. Furthermore, a fully-integrated system that can be operated in the field offers the potential to expand the use of STR analysis beyond an evidentiary role at trial to an investigative role at the crime scene. A fully integrated STR analysis system based on microfluidic biochip technology for forensic laboratory and field-forward operation will be described. This system comprises three modules to perform: (1) DNA purification and human specific DNA quantification, (2) multiplexed STR amplification, and (3) separation and detection of the resulting amplicons.

The development of a rapid PCR amplification module that can be applied to both conventional tube- and biochip-based systems will be reported. Rapid biochip based multiplex amplification is accomplished by a custom thermal cycler that heats and cools reaction solutions within an accompanying biochip at rates of approximately 15°C/sec. The single-use disposable biochip processes 16 samples simultaneously and is fabricated by injection molding. Following optimization of amplification reaction components and thermal cycling protocols, the system allows thermal cycling to generate full profiles with commercially available STR primer kits in approximately 17 minutes. Similar results are achieved in conventional tube reactions with a fast commercial thermal cycler. All

STR amplifications are characterized by separation and detection on a microfluidic biochip-based electrophoresis system, Genebench-FX™ Series 100.

Data will show the resulting STR profiles satisfy forensic interpretation guidelines for signal strength, inter-loci peak height balance, heterozygous peak height ratio, incomplete non-template nucleotide addition, and stutter. It will be demonstrated that rapid STR amplification can be utilized in forensic laboratories and that the biochip-based approach is well-suited for incorporation into a fully integrated microfluidic forensic DNA analysis system.

**STR Analysis, Multiplex PCR, Biochip**

## A178 Hazardous Materials and Environmental Crimes

*Elizabeth A. Mishalanie, PhD\*, United States Environmental Protection Agency, U.S. EPA/OECA /OCEFT/NEIC, Denver Federal Center, Building 25, Box 25227, Denver, CO 80225*

After attending this presentation, attendees will be informed about environmental forensic laboratory activities that support the U.S. EPA's criminal enforcement program.

This presentation will impact the forensic community by demonstrating the commonalities between environmental forensic laboratory operations and conventional forensic laboratory operations, and by identifying unique challenges in environmental forensics. It will also demonstrate that the U.S. EPA rigorously pursues knowing and willing violations of environmental laws that may have a substantial impact on human health, the environment, and the economy.

Three criminal cases will be reviewed to illustrate the selection and collection of physical evidence, laboratory examinations, criminal charges, and outcomes. These cases involve the improper removal of asbestos-containing materials from a school, the illegal disposal of 300 tons of chemicals, and the discharge of hazardous waste into a public sewer system which resulted in serious injury to a city employee. Legal challenges associated with environmental crime cases will also be presented.

Case #1 involves the use of dangerous practices to remove asbestos-containing materials (ACM) from a school. During and after the ACM removal, several hundred people were exposed to asbestos fibers. Evidence was collected from nearly 20 locations, and polarized light microscopy was used to identify and quantify asbestos fibers. Chrysotile asbestos was identified in most samples and was present at relatively high levels. Four individuals were charged with numerous violations of the Clean Air Act, Clean Water Act, Toxic Substances Control Act, and other charges including conspiracy, making false statements, and fraud.

Case #2 involves the mishandling of hazardous waste on an international level. In an attempt to dispose of surplus chemicals, a chemical brokerage business in the United States shipped numerous chemicals to an alleged buyer in Nigeria via Rotterdam. Upon arrival in Rotterdam, the Dutch government discovered that some of the containers were leaking. The purported Nigerian buyer could not be located, and in accordance with international law, the authorities did not permit the cargo to proceed to Nigeria. A team of investigators from the U.S. and the Netherlands collected evidence for two days. Materials sampled from drums were screened on site using portable X-ray fluorescence spectroscopy, acid/base indicators, and chemical spill classifiers. Laboratory examinations were pursued to evaluate the materials for

hazardous waste characteristics as defined by the U.S. hazardous waste regulations. These characteristics included ignitability, corrosivity, and toxicity. The laboratory tests revealed that several samples exhibited the ignitability or toxicity characteristics. The owner of the chemical brokerage company was charged with storing hazardous waste in the US without a permit, exporting hazardous waste outside the US without the consent of the receiving country, and transporting hazardous waste without manifests to un-permitted facilities.

Case #3 involves hazardous materials that were discharged into a public sewer system. An electroplating company used acids, bases, metal-containing solutions, and other hazardous chemicals in their production processes. The company attempted to treat their waste in a manner that overburdened their in-house treatment system which rendered the system ineffective. They diluted waste before discharging in an attempt to keep pollutants below permit limits and hired a company to flush their sewage lines to remove chemical sludge blockages resulting from dumping waste into the sewer system. Investigators collected evidence from the sewer and drain lines, pretreatment units, and process tanks to determine if the company violated its pretreatment permit. Several months after the investigation began, there was an incident involving a city employee who was seriously injured from exposure to hazardous vapors while monitoring the wastewater effluent from the company. After this incident, additional evidence was submitted to the environmental forensics laboratory. Other evidence collected during the search warrant execution provided chemical data used during case development. The company and the operations manager were charged with conspiracy to violate the Clean Water Act, making false statements, and negligent violation of the Clean Water Act.

These criminal cases are typical examples of environmental crimes pursued by the U.S. EPA. The Agency also pursues criminal enforcement of environmental laws involving drinking water, pesticides, importation and exportation of chemicals such as freons, and the release of hazardous chemicals on land and into the atmosphere.

**Environmental Forensics, Environmental Crimes, Hazardous Materials**

## A179 World Trade Center Dust Ground Zero and Beyond

*Nicholas D.K. Petraco, MS\*, John Jay College, 899 10th Avenue, New York, NY 10019*

The goal of this demonstration is to demonstrate the powerful nature of PLM as an analytical instrument in the solving of complex forensic and environmental analytical problems.

This presentation will impact the forensic community by emphasizing the importance of the continued use of polarized light microscopy in the forensic science laboratory.

On the morning of September 11, 2001, in New York City, the World Trade Center (WTC) financial complex was reduced to a fine powdery dust by two commercial passenger airplanes, flow by terrorists. Since this was a unique, cataclysmic event, an analytical method to quickly and accurately study the dust specimens had to be developed. Initial studies revealed that the dust generated by the collapse of the buildings was composed of a myriad of materials. It appeared that all the materials composing the buildings, and all of the buildings' contents were literally pulverized by the collapse of the Twin Towers. The complex nature of this material dictated the necessity to develop a PLM method to study

these WTC dust specimens. Aliquots of WTC dust specimens were taken at random from samples collected at Ground Zero and around the surrounding area and analyzed as follows: (1) each bulk specimen was thoroughly loosened and mixed gently using an agate mortar and pestle, (2) each bulk sample was equally divided into eight aliquots, (3) each aliquot was divided into eight equal portions, (4) each portion was placed on a microscope slide (MS), covered with a No. 1½, 22mm, round cover-glass, and dispersed evenly in Melt Mount® 1.539, and (5) each specimen was labeled for identification. Next, a quantitative particle count of each specimen was carried out with a PLM fitted was a Chaulkly, point-count reticle. At least 1,000 particles were counted for all of the microscope slide preparation made from each bulk specimen. The results were recorded on a WTC dust data sheets. This data was used to compute the percent of each material present in the average specimen. The findings of this study are presented in this paper.

**WTC Dust, PLM, Ground Zero**

## A180 Brake Pad Friction Particles: SEM-EDX Analysis and Comparisons to Gunshot Residue

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After attending this presentation, attendees will gain further insight into standard guidelines in the interpretation of gunshot residue.

This presentation will impact the forensic community by discussing the similarities of brake pad particles to gunshot residue.

Gunshot (primer) residue (GSR) analysis utilizing scanning electron microscopy/energy dispersive X-Ray spectroscopy (SEM/EDX) is the preferred method in Forensic Science. ASTM International issues a standard, E1588, which is the foundation document for the collection, analysis, and interpretation of SEM/EDX data, primarily on automated systems. In 2007 this standard underwent its required five year review. Two major changes were issued in the new document: (1) particles consisting only of the elemental triad, lead (Pb), barium (Ba) and antimony (Sb) are characteristic of GSR while Ba-Sb containing particles are no longer considered in that category, and (2) only trace levels of iron (Fe) are allowed in characteristic or consistent (Pb, Ba, Sb, Pb-Ba, Pb-Sb, Ba-Sb) particles. The impetus for the latter was based primarily upon published results of Torre, et.al. (*J. For. Sci.,* **2002**, 47(3), 494-512) in which particles attributed to automotive brake pads had chemical and morphological properties akin to GSR. The former revision is not a topic for this presentation.

The brake pad particle/GSR similarities have been broached by the defense in Court. While an experienced GSR analyst would not confuse the two species, the analyst will most likely not have observed such particles aside from the published images and spectra of Torre, et.al. To that end, we have undertaken a study of both rim and rotor particles from sixteen vehicles belonging to employees of the Harris County Medical Examiner's Office (HCMEO). Particles were obtained by gloved wipes of either left front rims or rotors . The sampling medium in all cases was aluminum stubs affixed with double-sided carbon tape. Each glove was sampled prior to wiping the surface of interest. The vehicles were all late-model (1996 to present). The analysis of these samples was performed via SEM/EDX was performed according to Standard Operating Procedures in place at the HCMEO using an ASPEX Corporation Personal SEM II. Automated classification was carried out during the analysis using ASPEX Corporation Automated Feature Analysis/GSR Reporter software. All particles classified as either characteristic of or consistent with GSR were reexamined in detail for confirmation of their chemical and morphological properties.

A total of 265,622 particles were classified during this experiment. Thirty-one characteristic (Pb-Ba-Sb) particles were classified but none was confirmed. Particles classified as consistent with GSR totaled 3,806 and only three (Sb) were confirmed. Eight Pb containing particles were detected but owing to significant Fe composition were not confirmed as per E1588-07. Over 75% of the particles classified fell into an iron-rich category.

Two striking features of these results are noted in that no characteristic particles were confirmed in this study and the paucity of Pb in the population. A discussion of these results in light of current knowledge of brake pad composition will be presented. A case study of GSR from a victim of an accidental firearm discharge will be presented.

**Gunshot Residue, Brake Pad, SEM-EDX**

## A181 Evidence to Consider When Evaluating Bullet Defects in Clothing for Characteristics of Entrance Versus Exit in Instances Where Distance Precludes Gunpowder Deposition

*Kay Sweeney, BS\*, KMS Forensics, Incorporated, PO Box 8580, Kirkland, WA 98034*

After attending this presentation, attendees will learn about physical evidence characteristics that can help in determining whether a bullet hole in clothing worn by a person suffering a gunshot wound is an entrance defect or an exit defect when the clothing does not support gunpowder deposits in the area of damage.

This presentation will impact the forensic community by demonstrating that the comprehensive examination and analysis of the bullet damaged clothing being worn by a shooting victim in mid to long-range distance shootings can contribute significantly to the determination of entrance versus exit sites.

Clothing items with bullets holes recovered from persons injured in shootings can be carefully evaluated for firearm discharge generated evidence (including transfer of lead to fabric at the site of bullet perforation) and damage characteristics, and conclusions regarding the differentiation between bullet entrance holes (damage) and bullet exit holes (damage) are possible.

Typically, in shootings where people are injured by bullets passing completely through the body, medical doctors examine the victims to determine a course of medical treatment for those still living or to determine cause and manner of death in the case of fatal injuries. In cases where the victim lives it is very likely that the attending physician has little or no experience at determining gunshot wounds for entry and exit characteristics so that type of determination my depend solely on an evaluation of clothing items worn by the victim. In cases where a forensic pathologist conducts an autopsy there tends to be considerable reliance on the interpretation of the wounds for evidence of entry or exit

by the autopsy surgeon, but those characteristics are not always clear cut and therefore confirmation may depend on an examination of clothing items.

When a live cartridge is fired, chemical compounds generated by the ignition of primer cap contents and gunpowder combine in a mixture of gases and particulate that produces a rapidly expanding pressure component behind the bullet, initially inside the cartridge case and eventually the chamber and barrel of the firearm, as the bullet is forced through the barrel of the firearm and out the muzzle. The bullet does not form a perfect seal inside the barrel of the firearm by any means and a significant amount of the expanding cloud of gases and particulate behind the bullet actually escapes past all sides of the bullet creating a coating of chemical residue on the exterior of the sides of the bullet. If the first target contacted by a fired bullet is clothing being worn by a person, then as the bullet passes through the clothing fabric, the chemical residue coating on the bullet transfers (at least partially) to the perimeter of the perforation damage in the clothing. This transfer is commonly referred to as "bullet wipe". Bullet wipe has been found to generally contain lead in levels well above normal environmental conditions.

This presentation reports on the results of testing conducted thus far involving one 9mm semi-automatic pistol using nine different rounds of 9mm Luger ammunition representing seven manufacturers or brands.

In order to establish baseline information relating to the source of lead in bullet wipe patterns on clothing, the gunpowder, jacketed bullet and cartridge case of one round representing each of the seven manufacturers were tested using x-ray fluorescence spectrophotometry (XRF). All gun powders were found to contain lead ranging from 25 ppm to 180 ppm.

One each of the seven representative manufacturer's cartridge cases with the live primer cap in place was fired in the 9mm pistol while aimed into white, 100% cotton t-shirt fabric at a muzzle to target distance of four inches. The resulting smoke and particulate deposit on the white cotton fabric was tested for lead using XRF. Lead content was noted in the range of from 7,000 parts per million (ppm) to 19,000 parts per million in the deposit. Copper, antimony, mercury and zinc were also noted in significant quantities.

The nine representative rounds of 9mm Luger ammunition were fired into 100% white cotton t-shirts, fitted over a device to approximate body torso thickness, at a muzzle to target distance of six feet (72 inches) in a manner that created an entrance hole in the front and an exit hole in the back of the t-shirt for each shot. All nine bullet entrance holes and exit holes were subjected to XRF analysis. Entrance hole residues ranged in lead concentration levels from 190 parts per million (ppm) to over 1,000 ppm while exit hole residue lead levels ranged from zero to 74 ppm. In all cases, entrance hole lead concentrations were at least six times higher than exit hole lead levels.

A second test firing was conducted where-in a white 100% cotton long sleeved shirt was place over a white 100% cotton t-shirt, which had been placed over a device as described above. Five rounds of different ammunition selected from the original group of nine 9mm rounds were fired into the shirts at a six foot muzzle to target distance such that entrance and exits holes were created in the front and back respectively for every shot. All five primary bullet entrance holes and all exit holes in the exterior white long sleeved shirt were subjected to XRF analysis, as were all five secondary entrance holes and exit holes in the t-shirt (undershirt). Primary entrance hole residues in the long sleeved (outer) shirt ranged in lead concentration levels from 114 parts per million (ppm) to nearly 500 ppm while exit hole residue lead levels ranged from zero ppm to 74 ppm. Secondary entrance holes in the t-shirt (undershirt)

supported residues with lead levels ranging from 34 ppm to 250 ppm. The percentage of loss in lead levels identified in bullet hole residues between the primary entrance and secondary entrance sites ranged from a low of 36% and a high of 70%.

Damage characteristics exhibited at bullet perforation sites in clothing can also be an indicator for assessing the likelihood of entrance or exit. At an entrance site the clothing is usually supported by body mass and allows for a fairly well defined, circular hole while fabric at the exit site is often not supported and the exiting bullet tends to tear the fabric rather than create a clean hole. Bullet damage characteristics in clothing are best evaluated very cautiously and should always be considered in the context of other evidence.

Firearm discharge is a violent and powerful action and in some instances fragments separate from the fired bullet and can be lodged in the clothing near the bullet entry site. A careful microscopic search may reveal the presence of such particulate at the site of bullet entry.

When a fired bullet exits a person's body it will generally bring with it body tissue fragments and these fragments will be deposited as tissue bits, bone fragments and body fluid spatter on the next nearest target. When there is clothing covering the exit site the tissue/bone/fluid deposit pattern may be significant enough to help in the determination of entrance versus exit injuries. Tissue/bone/fluid deposit patterns must again be cautiously evaluated in the context of other areas of body fluid deposits, types and locations of other injuries, orientation of the significant deposits and evidence handling history.

Clothing items with bullets holes, body tissue deposits and bullet fragments as recovered from shooting victims can be examined and analyzed for the chemical content of their "bullet wipe" deposit patterns and other physical evidence characteristics to successfully identify which is an entrance and which is an exit defect.

**Bullet Entrance, Bullet Exit, Clothing Examination**

## A182 Detection of Molecular Markers for the Identification of Gunshot Residues by Solid Phase Micro Extraction - Gas Chromatography/Nitrogen Phosphorous Detector (SPME-GC/NPD)

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After attending this presentation, attendees will have the opportunity to discuss our new method for the detection of trace amounts of methyl centralite and ethyl centralite from the GSR collection kit with a novel extraction scheme of using solid phase micro extraction. The ease of adaptation of this technique to forensic labs from other chemistry-focused areas will be shown. Discussion of similar efforts towards advances in science being applied to forensics will be encouraged.

This presentation will impact the forensic community by exploring the many compounds that are specific to gun powder primers and stabilizers. For the purposes of uniqueness, methyl centralite and ethyl centralite were reported as highly significant to GSR. These two stabilizers are commonly used in virtually all ammunitions and are not typically found in normal environments. Detection of trace amount of methyl centralite and ethyl centralite has been a challenging task. Our investigation of a novel extraction technique has created an alternative

way to detect significant molecules form GSR related samples. The new method will have a great impact on the determination of molecular marks for those GSR related samples that couldn't be easily determined by conventional analytical procedures.

The confirmative detection of gunshot residues (GSR) relies on the use of scanning electron microscope - X-ray energy dispersive spectrum (SEM-EDX) to identify micrometer-sized particles that have the characteristics similar to known GSR-particles. This widely accepted method can determine the morphology, as well as the inorganic chemical composition, of the GSR-like particles. Collection of these micrometer-sized particles from samples is commonly achieved by using a standard GSR collection kit, which is a carbon double-sided tape sticking on an aluminum disc. To reduce false positive and false negative results for the detection of GSR, identification of organic components in GSR is a feasible way to provide a second layer of information. The idea in this work was to develop a complementary analytical method to pick up signature organic molecules collected on the GSR collection kit.

Methyl centralite (MC) and ethyl centralite (EC) are two important molecular markers for a positive identification of GSR, even though not all ammunitions contain these two compounds as stabilizers. The presence of these two molecules from the sample would highly suggest the presence of GSR. A sensitive extraction scheme to extract MC and EC from the samples collected by standard GSR collection kits has successfully been developed. The extraction was achieved using a solid phase micro extraction (SPME) technique. The SPME fiber was exposed to the head space in a 20-mL vial that contained the sample. The vial was also dipped in an oil bath maintained at 80 degrees Celsius during extraction. With the increase of extraction time, there was no impact on the extraction of MC. However, extraction of EC was increased when extraction time was increased. After extraction, the extracts were then desorbed in a GC injection port at 280°Celsius for 5 mins and splitlessly injected to a gas chromatography (GC) coupled to a nitrogen phosphorus detector (NPD) for analysis. Twenty ng of EC and MC spiked onto a GSR collection kit were readily detected under the optimal condition. No interference peaks were overlapped with EC peak at retention time of 16.7 mins. Unfortunately, one interference peak slightly overlapped with MC at retention time 15.8 mins.

This new extraction scheme can be easily applied to the extraction of MC and EC from different GSR collection methods, such as cotton swabs. Further research will also focus on designing an efficient extraction camber specialized for the extraction. The detection of sub nano grams of MC and EC from the sample may be possible in the near future.

**Solid Phase Micro Extraction, Gunshot Residues, Trace Evidence**

## A183 Virtual Forensic Laboratories: A New Instructional Tool

*Richard Saferstein, PhD*, Forensic Science Consultant, 20 Forrest Court, Mount Laurel, NJ 08054*

After attending this presentation, attendees will be familiar with recently developed virtual laboratories designed to be used in a 2 or 4-year college offering an introductory criminalistics or forensic science course.

This presentation will impact the forensic community by its design to improve the teaching of introductory criminalistics by familiarizing the student with state-of-the art laboratory protocols.

Thirteen virtual forensic laboratories have been created to capture the real-life experience of working in a forensic lab. The targeted audience is students enrolled in undergraduate forensic courses and programs. The labs were developed with Flash 8 and use 2-dimensional art to simulate the lab setup. Each lab starts with a crime scene scenario. Typically, a lab will have different sub-sections designed to take the user through a logical flow of the different components of the experiment. The user will be allowed to interact with equipment simulations while performing all the steps in an experiment. The user will also be allowed to fill datasheets while performing the experiment.

Two of the virtual experiments, a forensic hair comparison and a drug analysis utilizing gas chromatography-mass spectrometry, will be used to illustrate the utility of virtual laboratories as an instructional tool in the classroom.

**Virtual Laboratory, Virtual Hair Comparison, Virtual Drug Analysis Drug**

## A184 Spark - Induced Breakdown Spectroscopy (SIBS) Analysis of Bioaerosols and Biological Warfare Agents

*Morgan Steele, MS*, 2895 Cherry Way, Parker, CO 80138*

After attending this presentation, attendees will be introduced to a new device and method for air sampling and analyzing harmful biological aerosols. After attending this presentation, participants will understand the principles of Spark-Induced Breakdown Spectroscopy (SIBS), the equipment and techniques used, and how SIBS compares to Laser-Induced Breakdown Spectroscopy (LIBS). The aim of this work was to investigate further use of SIBS for bioaerosols as an alternative to the more expensive and well-known LIBS.

This presentation will impact the forensic community by introducing a unique method for rapidly screening and analyzing biological warfare agents as airborne pathogens. SIBS has the ability to distinguish between atomic spectra of airborne biological particles. These spectra can then be used to differentiate between harmful and harmless biological species, such as Bacillus anthracis (Anthrax) vs. Bacillus thuringiensis (Bt). Therefore, SIBS can be used as a real-time trigger sensor for biological warfare agents.

SIBS was initially developed as a real-time sensor for toxic heavy metals in aerosols at detection limits of 1 to 10 mg/m$^3$. SIBS uses a high-energy electrical spark between two electrodes to ionize, vaporize and excite the elements of the sample of interest. The aerosol sample passes through the spark gap containing rod-shaped electrodes, which ablates the sample creating plasma. Within this plasma, the ablated material dissociates into ionic and atomic species. After plasma cooling, atomic and molecular emission lines of the sample can be observed. The SIBS apparatus is coupled with a Czerny-Turner Spectrometer and an Andor iCCD detector. The more familiar technique LIBS, on the other hand, is very similar, except that it uses a focused laser pulse as the excitation source. When the highly energetic laser is discharged, it ablates a small amount of the sample and creates plasma.

In this experiment, biological warfare simulants Bt, Ragweed Pollen, and Johnson Grass Smut aerosols were studied in the spectral regions around 380nm. These samples were made into an aqueous suspension and sprayed through a nozzle to create an aerosol standard. Aerosolized DI water and electrode material alone were also studied. The electrodes were originally placed so that the focus of the optics covered the

energized side of the gap. The gap was then translated to allow optical probing of the center of the plasma. Finally, the gap was moved further until the emission at the ground side of the gap was in view of the collection optics. In this presentation, we will present data acquired in each of the three locations at a variety of delay times. This presentation will also include unique molecular features found in this spectral region.

The results of this and other studies demonstrate that SIBS can spectrally distinguish between biological and non-biological samples, as well as distinguish between biological samples within the same species. The low detection limit, sensitivity, and discrimination potential of SIBS indicates this system as an alternative to the costly LIBS system. In the future, SIBS could also be useful in other areas of forensics such as trace and drug analysis.

**SIBS, Bioaerosols, Air Sampling**

## A185 Analysis and Discrimination of Electrical Tape Backings by FTIR and py-GC/MS

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After attending this presentation, the attendees will be aware of current FBI protocols for analysis of electrical tapes and the common chemical compositions thereof.

This presentation will impact the forensic community by providing a better understanding of the relative discrimination powers of forensic analyses of electrical tape backings using Fourier transform infrared spectroscopy (FTIR) and pyrolysis-gas chromatography-mass spectrometry (py-GC/MS).

Electrical tapes are often submitted to crime laboratories as evidence associated with improvised explosive devices or other violent crimes. The FBI Laboratory performs comparative electrical tape examinations to explore the possibility of an evidentiary link between a suspect and a crime or between different crime scenes.

Submitted samples are first evaluated by visual and microscopic means to evaluate physical characteristics such as backing color, adhesive color, width, degree of gloss, surface texture, and thickness.

If the samples are consistent following visual and microscopic examinations, chemical composition of the tape adhesive and backing is evaluated. Current FBI protocol calls first for chemical analysis via FTIR with a microscope attachment. FTIR analysis can provide information regarding the rubber and polymeric materials used to formulate a tape's adhesive and backing as well as some information for the plasticizers and flame retardants that are present. However, typically a significant amount of peak overlap occurs, making spectral interpretation difficult. Therefore, in most instances the individual chemical constituents are categorized into general classes rather than identified. For samples that cannot be differentiated by FTIR examination, scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) is then performed to compare elemental composition. Finally, py-GC/MS is performed on each component if samples have yet to be discriminated. This technique breaks the organic components down, separates them, and provides more conclusive information as to the identity of the chemical constituents. As a result, py-GC/MS is particularly useful in identifying

the rubber component(s), the type(s) of plasticizers, and any other organic additives present.

This study involved the analysis of ninety electrical tape samples utilizing the current FBI Laboratory protocol. Most of the tapes were purchased by FBI personnel at discount stores or home-improvement retailers, are marketed as general purpose or economy grade, and cover a variety of U.S. and foreign manufacturers. Therefore, the sample set represents tapes that could be easily obtained by consumers and would be comparable to casework evidence submitted to the FBI Laboratory.

This project was designed with a number of objectives in mind. They include: (1) determination of the range of physical characteristics and chemical compositions of electrical tapes, (2) evaluation of the ability of the individual techniques to discriminate samples, and (3) assessment of the ability of the overall analytical scheme to distinguish between samples. The analysis and discrimination of electrical tape adhesives was the topic of a previous presentation. The subject of this presentation will be the composition of the electrical tape backings as determined by FTIR and py-GC/MS. Furthermore, a comparison of the two techniques' discrimination ability will be discussed.

**Electrical tape, FTIR, py-GC/MS**

## A186 An Evaluation of Pyrolysis Gas Chromatography/Mass Spectrometry and Summed Ion Profile Library Matching for the Classification and Identification of Wood Samples

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After attending this presentation, attendees will have an understanding of the advantages and disadvantages of using pyrolysis gas chromatography/mass spectrometry for the characterization of various types of wood.

This presentation will impact the forensic community by providing some initial insight into a new method for the analysis of wood samples. As this is a preliminary attempt at using this technique in this fashion, it is hoped that others will pick up where this project leaves off and pursue additional research.

Wood, briefly defined, is the solid fibrous material found under the bark of trees. It is a natural material that has a multitude of uses making it a fairly ubiquitous substance within any environment. It should therefore be no surprise that wood, in any of its various forms, is routinely encountered during trace evidentiary examinations. Whether it is submitted as large fragments from structures, splinters from fragmentations, or sawdust, it has the potential for being a very informative type of evidence. In all of the forms encountered, the identification of the specific kind of wood from which the samples originated can be utilized to provide important investigative and/or comparative information to assist with casework.

Traditional means for classification and identification of types of wood rely heavily upon visual and microscopic analysis whereby identifications are made based upon recognition and comparison of physical and structural characteristics that are imparted to the wood during growth. This is usually carried out by a skilled analyst who possesses a large amount of knowledge and training pertaining to the recognition of various characteristics of different types of wood. In any

case, analysis by such means is by far the best course of action to take when attempting to identify wood. However, such examinations are best carried out with relatively large samples, which may or may not be available during the average case. In such a circumstance it may be important to substantiate any potential identification with some additional data.

Wood is not only physically/morphologically complex, it is also quite rich in chemical content. In addition to the primary chemical constituents of wood (e.g., cellulose), various additional compounds are known to be present in smaller amounts. As an example, it is well known that soft woods often contain a variety of terpenes including but not limited to a-pinene, b-pinene, and limonene. It was the objective of this study to investigate the possibility of classifying and identifying various different types of woods according to potential differences in their chemical composition.

For the purpose of this project duplicate samples of 54 common woods at a size of approximately 0.5mm x 0.5mm were placed in quartz sample tubes and analyzed using pyrolysis gas chromatography-mass spectrometry. A thirty-minute run time was used with pyrolysis occurring at 700°C for 20 seconds and oven conditions beginning at 40°C and peaking at 300°C at a ramp of 12° per minute. This process separated the pyrolyzed chemical components of the very small samples of wood enabling the identification some of these components. The resulting pyrograms were compared for overlapping patterns and examined for specific chemical content. In order to achieve these analyses extracted ion profiling was performed for various classes of compounds and an in-house library was prepared for sample comparison via summed ion profiles.

The project produced some promising results and it appears that the methods employed may be useful for the identification of wood samples. Sample preparation is simple, analysis times are short, and a large amount of information can be obtained from a relatively small sample. Based on these attributes, this method could be very useful in the identification of wood in forensic casework. However, it should be noted that there is currently no substitution for visual and microscopic means of wood identification. The results of this study would best be utilized to support information provided by more traditional types of analysis.

**Trace Evidence, Wood, Pyrolysis Gas Chromatography/ Mass Spectrometry**

## A187   The Bad Breath Rapist

*Beth A. Saucier, BS, BS\*, Massachusetts State Police Crime Laboratory, 124 Acton Street, Maynard, MA 01754*

After attending this presentation, attendees will obtain knowledge about the unusual circumstances in a home invasion and sexual assault case. The initial investigation was based on the victim's statements and what she smelled at the time of the attack. The Massachusetts State Police Crime Laboratory assisted the local police department during their investigation using the Criminalistics and DNA examinations.

This presentation will impact the forensic community by presenting actual case information and discussion on the challenges encountered during the Criminalistics Department's processing of evidence in this case. It also will provide information on how evidence should flow through an examination process. Interesting case facts will also be discussed and questions will be addressed.

On February 3, 2005, at approximately 12:30 a.m., an armed home invasion and sexual assault occurred on Elm Avenue, a residential neighborhood, in Quincy, MA. The victim was grabbed from behind after coming out of the bathroom after freshening up after a long day of work. A knife, which was smaller than a sushi knife, was put to her throat by her attacker and she was then thrown to the floor. A struggle ensued. Her mouth was duct taped and she was tied to the bed with plastic zip ties and duct tape. She was then sexually assaulted and the suspect left the premises with money taken from her purse. Her boyfriend came home to find her still tied to the bed in a prone position. The police were called and the investigation began. During the investigation, the victim was asked if she could identify her assailant. Due to the fact that he wore a ski mask and was completely dressed in black, she stated that she could not identify his face but she could identify his smell. He was breathing very heavy and she recognized his bad breath – it smelled very familiar!

The Massachusetts State Police Crime Lab was contacted and immediately responded to the scene of the crime to collect any biological and trace materials that were present. Samples were collected from the scene - clothing and duct tape, among other items, and the victim herself through the means of a Sexual Assault Evidence Collection Kit. The items collected were processed in the Criminalistics and DNA units. Processing of items, challenges encountered and results of analyses will be discussed. The results of the analyses completed in this case helped solidify the investigation by the Police Department. The case did go to trial in the Norfolk County Superior Court in Dedham, MA, testimony was provided and an outcome was determined.

**Home Invasion, Sexual Assault, Case Study**

## A188   Human Breath Analysis as a Forensic Tool: Detecting Prior Location and Suspect Activity of a Subject

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After attending this presentation, attendees will be familiar with the concept and applications of human breath analysis. They will also learn methods in which breath analysis can be applied to a forensic setting, specifically identifying the prior location or activity of an individual.

This presentation will impact the forensic community by highlighting a new research tool that may aid in linking an individual with a location, as well as possibly identify subjects working with illicit materials, e.g., drugs or explosives, before harm is done to humanity.

This presentation will demonstrate the use of human breath analysis using gas chromatography-mass spectrometry to detect chemical compounds present in breath in trace quantities. These compounds can provide information about the prior location or prior activity of a subject.

Identification of the prior location or activities of an individual can have important ramifications in the forensic community. A method that

could identify if or when a subject was in a particular location would aid in associating a suspect with a crime scene. The ability to detect compounds on human breath that would indicate illicit activity, such as drug or explosive synthesis, would allow suspects to be identified early in the synthetic process and perhaps prevent distribution or a terrorist event.

All locations have a chemical signature caused by the compounds present in the air due to natural compounds, outgassing of materials, and the activities pursued in the space. For example, the air in a laboratory used to synthesize drugs will contain chemicals used in that synthesis which are not commonly found in a typical air sample. An individual in the location is consequently exposed to these compounds, generally present in very low concentrations. As an individual breathes, these compounds are drawn into the lungs and pass into the bloodstream via the pulmonary alveolar membrane. After leaving that location, the compounds are eliminated from the body at different rates and through different excretory systems; however, a significant portion of these compounds are eliminated through exhaling. Therefore, it was hypothesized that by collecting and analyzing human breath, these compounds can be detected and may provide information about the history of the subject.

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze human breath samples collected before and after a series of exposures. Breath samples were obtained before and after a subject visited a hardware store and nail salon, and at several different locations. Chemical signatures were detected in these breath samples and traced back to a particular compound present in the air at each location, connecting the individual with a particular site. Breath samples were also obtained before and after controlled chemical exposures in the laboratory, including oral exposure to pseudoephedrine HCl, a common starting material in the synthesis of methamphetamine, and inhalation exposure to hexamine, a common starting material in the synthesis of explosives. The presence of these compounds was detected and monitored in breath samples for hours after exposure. As these experiments were performed on the small scale in a controlled environment, it is thought that real-world subjects would have an even higher level of exposure and would allow for these signatures to be detected over longer time courses.

**Breath Analysis, GC - MS, Suspect Activity**

## A189  DNA Recovery From Fired and Unfired Cartridges

*Stacie R. Kaufman, BS\*, Lawrence Quarino, PhD, and Brian J. Gestring, MS, Cedar Crest College, Department of Chemistry & Physical Science, 100 College Avenue, Allentown, PA 18104*

The goal of this presentation is to show the extent of DNA degradation on fired cartridges and whether sufficient DNA can be recovered from a fired cartridge in order to obtain meaningful DNA typing results.

This presentation will impact the forensic community by providing new insight to the evaluation and analysis of evidence collected from a shooting scene. Often, investigations involving shootings do not result in the recovery of a firearm or a suspect may not be tied to a scene or a victim by traditional means. In this case, any cellular material deposited on ejected cartridge cases during the process of loading and handling the firearm may be capable of providing additional investigative information

if DNA can be extracted from the cartridge. Despite problems with stochastic effects and allelic dropout, laboratories with the capability of typing low copy number DNA continually provide evidence that DNA profiles can be obtained from "touch" samples.

DNA quantities from various sources were placed on cartridges and swabbings of test areas were taken before and after firing. A percent loss of DNA was determined on each cartridge using an *Alu*-based real time quantitative PCR assay for human DNA. In the initial stages of this study, 37 neat blood samples (2ul volume) yielded a mean DNA quantitation value of 4.63ng/uL recovered before firing and a mean value of 0.14ng/uL after firing, showing a 97% loss of DNA. Data obtained utilizing 12 blood sample diluted 1:10 (2ul volume) recovered a mean DNA quantitation value of 0.35ng/uL before firing and 0.20ng/uL after firing, showing a 45% loss. In addition, samples were typed using PowerPlex®16 (Promega), and the number of alleles lost during firing were determined for each cartridge.

There are many factors which may influence the ability to detect DNA from an ejected cartridge casing. This includes the weapon type, ammunition used, caliber of the weapon used and the temperature within the firing chamber. Within the firearm any DNA adhering due to the handling of the magazine and loading the cartridges may be subjected to an array of temperatures which may degrade and denature any DNA originally present. The possible role that each of these factors play in the degradation of DNA during the firing process will also be discussed.

Successful DNA recovery may also be dependent on the location of the cartridge sampled as well as the medium used for swabbing. This study investigated several extraction techniques as well as various swabbing substrates in their ability to collect DNA from low copy samples. Previous research has determined that a polyester felt, used in place of a cotton swab for recovering DNA, works efficiently when employing a low copy number extraction protocol. The optimal extraction technique combined with an effective swabbing substrate allows for greater recovery of DNA samples of small quantities.

Preliminary results suggest that the small number of epithelial cells deposited through transfer and touch may produce genetic DNA profiles attributable to an individual. The recent advances in DNA technology and the immense growth of forensic science have allowed DNA samples such as these to become a more important source of physical evidence.

**DNA Recovery, Fired Cartridges, Low Copy DNA**

## B1 Evidence From Explosives Correlated With Digital Evidence Examinations

Carrie M. Whitcomb, MSFS*, National Center for Forensic Science, c/o University of Central FL, PO Box 162367, Orlando, FL 32816-2367

After attending this presentation, attendees will see how high explosives create large amounts of evidence in small fragments and the manner in which the explosive scene is triaged can have a correlation with the large amounts of data in digital evidence coming into laboratories for processing.

This presentation will impact the forensic community by showing that it is all about process. Forensic processes come and go as new evidence evolves. The theory of certain processes can be applicable to various diverse forms of evidence that seem to have nothing in common. However, the desired outcomes are the same, find the most probative evidence as quickly as possible.

When I participated in the evidence collection and processing post-blast scenes resulting from an Improvised Explosive Device (IED) for the U.S. Postal Inspection Service, it was the rule that "everything" within the scene would be brought to the laboratory for examination. The vast majority of IEDs encountered utilized low explosives. The fragments were large and the damage to the environment varied. However, when an IED utilizing a high explosive detonates, the volume of debris from the environment of the crime scene is tremendous. A new approach for evidence collection and the redefining of "everything" are necessary.

Bombing scenes are very much like hard drives; bomb scenes are divided into grids and hard drives are divided into sectors. Each grid and each sector are evaluated to determine the presence of environmental debris and the presence of evidence. The solution for the high explosives crime scenes processing may be a clue to working a large digital evidence case with terabytes of data. Stay tuned!

**Digital Evidence, Large Volumes, Processes**

## B2 Standardization of Digital Forensic ResearchTechniques

Carey R. Murphey, PhD*, White Oak Labs, 5121 Valerie Street, Bellaire, TX 77401

After attending this presentation, attendees will have increased insight into the challenges regarding standardization of emerging techniques and protocols in the field of digital forensics and will be better able to evaluate various ways in which the research community might have greater impact on standard operating procedures used by labs or investigators.

This presentation will impact the forensic science community by identifying, clarifying, and analyzing issues observed in the process of translating published techniques into adopted standard operating procedures in digital forensics.

This presentation explores the challenges of taking a technique from peer-review and publication to use in standard operating procedures by labs and investigators. Also discussed are the various constraints related to the age of the field, vendor support, and the standardization and certifications processes that are intended, in part, to directly support widespread adoption of good practices.

A case study involving forensic examination of Windows® logs is used for illustrative purposes. Despite the combination of a peer-reviewed, published protocol and a freely available software tool that facilitates implementation of the protocol, the recommended approach appears to have had quite limited adoption in the form of standard operating procedures. This was unexpected and indicates additional requirements that appear to significantly impact adoption of good practices in digital forensic labs. Peer-review, freely available tools, and even standardization of good practices might have enhanced impact on adopted standard operating procedures if additional requirements of digital forensics labs are met.

Ultimately this presentation addresses the question, what can one do to encourage regional digital forensic labs or individual investigators to adopt peer-reviewed techniques for digital forensics? Peer-review of a protocol may be valuable support for satisfying *Daubert* challenges, but it is only one of a number of requirements that labs may face in order to adopt a protocol into its standard operating procedures. Many digital forensic labs have a strong reliance on commercial software tools, such that availability of a tool that supports the protocol is an important consideration for incorporating the protocol into standard operating procedures. In some ways, this may be inherent to digital forensics due to rapidly emerging information technology and aspects of commercial software tool development. Software tools can help satisfy requirements for reliability, reproducibility or uniform accuracy. In this opinion, even the combination of peer-review of a protocol together with a freely available software tool may still have quite limited impact. This can be seen in the contrast between the reliance on commercial tools in many labs compared with the more limited adoption of open source tools. Some labs may be reluctant to codify a standard procedure without associated commercial vendor support. This suggests that peer-review, tools, and even standardization efforts may have a significantly enhanced impact if additional requirements are met.

**Digital Forensics, Standard Operating Procedures, Requirements**

## B3 Understanding the Costs of Conducting Computer Forensic Examinations

Douglas G. Elrick, BA*, Digital Intelligence, 17165 West Glendale Drive, New Berlin, WI 53151

After attending this presentation, attendees will be able to accurately evaluate, compute, and budget the needed costs of digital forensic examiners. Whether starting a new unit or expanding an existing one, costs can be effectively estimated.

This presentation will impact the forensic community by providing managers and directors an introduction to the computer technology and practices of this forensic discipline, a guide for budgeting and planning to adequately equipment, and examples of the types of cases examiners will face.

Attendees of this presentation will learn about real costs associated with computer forensic examinations and will come away with an understanding and appreciation for the necessary types hardware, software, facilities, and training requirements for this newly recognized scientific discipline. A comparison of the commonly used forensic applications and hardware will be given. This will be helpful in the purchasing and budgetary development process for both managers and experienced practitioners.

Costs for conducting computer forensic examinations can be broken down into four main categories; hardware, software, facilities, and training. While these categories are similar in other forensic disciplines, the need for continuing updates is more apparent and pronounced in the computer field. Typical hardware and software startup costs requirements based upon a two-person section will range between $30,000 and $100,000. This presentation will highlight this range and describe the factors involved in the cost differentiations. Specific software programs and hardware devices will be addressed in a manner to present a perspective and comparison of the many features. A listing of what is considered "industry standard" applications based upon functionality will be provided along with a variety of lesser known and often less expensive or free alternatives. What must also be factored into the overall cost will be the need for annual updates of licenses and hardware changes. Many of the current forensic software packages are offering (some are requiring) annual subscription services for their products in order to receive updates and fixes. Hardware lifecycles are running approximately three years before upgrades are necessary. New types of storage media is a big reason for required upgrades. For example, when new interfaces to hard drives or new flash media types are developed the forensic workstation must be capable of connecting to it. This can often be accomplished through relatively inexpensive adapters. In other circumstances, it may require complete upgrades to the internal components of the computer. Approximately $10,000 a year may be necessary to cover these updates.

With regard to the facilities needed for digital forensics, while the data collection and inventory of the physical components of the submitted evidence are done in a traditional laboratory environment with the ability to address any chemical or biological contamination, the forensic computer analysis is typically accomplished at a desk location. The desk location should still be a part of the laboratory with all of its security and controls, but should be in an isolated place. Due to the nature of the data displayed, which is often child pornography, the examination should be conducted in an area where viewing is limited to the examiner and not to any passerby. The examination may also require considerable concentration and the work area should allow for minimal distraction.

Unlike most other forensic disciplines where the methods of analysis and identification are continually improving but the evidence itself has remained the same, with computer information the form of the evidence is consistently changing and evolving. This frequent change necessitates updated training. Continuing education is essential in order for examiners to stay abreast of new technologies and methodologies. Software and hardware providers offer regularly scheduled training updates and there are several computer forensic associations that provide methodology training each year. For state and local law enforcement grant-funded training opportunities are available. A minimum number of hours should be twenty hours per year, per examiner, as this meets the educational requirements of several industry certifications. This would provide the most basic of updates. A preferred number would be 80 hours, budgeted at $20,000 a year for two examiners.

Managers and directors who are not familiar with the computer technology and practices of this forensic discipline will have a guide for budgeting and planning to adequately equipment and provide for the types of cases examiners will face. Experienced examiners will be presented with an analysis of commonly used programs, hardware and training options.

**Computer Forensics, Budget, Costs**

## B4 A Case Study: Overcoming Anti-Forensic Methods Used on External Storage Drives

*Michael Andrew\*, CyberSecurity Institute, 21816 132nd Street Southeast, Monroe, WA 98272; and Steven Hailey, CyberSecurity Institute, 17716 Trombley Road, Snohomish, WA 98290*

This presentation is based on a case study involving theft of proprietary data and efforts to conceal the offense. After attending this presentation, attendees will be able to identify and overcome certain efforts made to mislead and frustrate forensic analysis of file system activities on external storage drives.

This presentation will impact the digital forensic science community by providing analysts a methodology and practical technique that will assist in accurate analysis of data stored on external hard drives.

There are three primary learning objectives for this presentation: (1) facilitate analysis of external storage drives that have been used with a computer running a Microsoft Windows operating system that utilizes the NTFS file system, (2) identify and interpret certain data artifacts recorded on an external storage drive by the operating system, and (3) utilize these artifacts and overcome anti-forensic methods, assisting in the accurate analysis of file creation, deleting, copying, and moving processes.

This case study outlines a methodology that can be used to detect the manipulation of creation date and time stamps associated with files copied to an external storage drive. The case study also presents a process that can be used to track the movement of files to and from an external drive without reliance on recovery of latent data, ( i.e., relevant file data and meta-data), or access to records located on the computer system that was used to copy the files onto the external drive.

The case concerns a large quantity of proprietary data that was downloaded to an external USB storage drive by employees, prior to departing a company. Analysis of records located in the USBSTOR sub-key on computers at the company revealed the date that the external drive was connected and used to copy the proprietary data.

In response to a court order, the defendants presented an external USB hard drive for analysis. The defendants refused to make available any computers that had been used to access the surrendered USB drive. They maintained they had never copied the proprietary data onto their personal computers and that their personal data was always kept separate from the downloaded data.

Analysis of the USB storage drive revealed the presence of proprietary files with creation date and time stamps that appeared to correlate with the connection records recovered from the USBSTOR sub-key on the company computers. However, further analysis revealed that the date and time had been manipulated at least three times during file creation processes, indicating multiple attempts to mislead and frustrate the analysis. The deception was discovered through analysis of artifacts on the USB drive that were generated as part of the System Restore function used by certain Microsoft Windows operating systems. Consequently, analysis was able to show that the presented drive was doctored in an attempt to make it appear as though it was the drive used to originally download proprietary data at the company.

Inspection of the artifacts also revealed that other relevant proprietary data had once been present on the drive, despite the claims of the defendants. The analysis was able to track the movement of these undisclosed files onto and off of the USB drive, demonstrating that the defendants had misrepresented their actions regarding the proprietary data and their compliance with the order of the court.

This case study is centered on a situation that presents significant challenges to an analyst; an external storage device is presented for analysis and the analyst does not have access to the computer that created the data on the storage device. The analyst cannot inspect records on the connected system to check if the file creation and file access date and time stamps for data on the storage device - derived from the system time

set on the connected computer - have been manipulated. The methodology used in this case will be beneficial to the analyst in these types of situations, and can provide independent verification of activities surrounding the data on an external storage drive or device.

**Anti-Forensics, Storage Drive, Time-Stamp**

## B5    A Virtual Architecture for Digital Forensic Tool Validation

*J. Philip Craiger, PhD\*, Chris Marberry, BS, Greg Dorn, BS, and Scott Conrad, BS, National Center for Forensic Science, University of Central Florida, PO Box 162367, Orlando, FL 32816-2367*

After attending this presentation, attendees will have an overview of virtual systems, tool validation, and how the two can be combined to create a powerful testing architecture.

This presentation will impact the forensic community by demonstrating how crucial it is that examiners validate their tools. This talk will provide examiners with the necessary information for them to create a comprehensive architecture for tool validation.

The evolving nature of computer software is fast paced and constantly changing. However, no software is perfect and anomalies can present themselves; bugs can be introduced with new features, or as an unintended consequence of a bug fix. This is, unfortunately, the reality of computer software and as a result of this it is vital for computer forensic examiners to validate the forensic tools they use and to ensure that the tool's results are accurate. A simple way to validate a tool is to compare a tool's calculated value against a previously known value, such as a one-way cryptographic hash value of a drive, volume, or even a file. Another is to "triangulate" results by testing the same function of several different programs against the same medium to see if they produce the same results.

While evaluating digital forensic proficiency tests it was noted several examiner's test results differed from our "validated answers." (The validated answers were obtained by triangulating results from several different forensic suites and different versions of these suites). Of interest was determining whether these discrepancies were due to user error, forensic suite error, or some other unanticipated anomaly (e.g., bad hardware).

In order to test these hypotheses a virtual architecture was developed that allows the separation of the influences of different forensic suites, different versions of the suites, and operating systems in order to identify the possible source of these errors. A typical validation methodology would involve dedicating several computers to running different forensic suites (separate computers would be used so as not to contaminate the results by installing and/or running two forensic suites on the same computer). This methodology results in significant duplicated time and effort for every single instance of forensic suite that requires validation. Disk imaging has simplified this process to a degree, by allowing a "baseline" image to be created and kept, but it is still undesirable in terms of required storage space and the amount of time required. Virtualization technologies, however, allow a significant portion of this process to be streamlined, in terms of both required disk space and time spent. One important feature that virtualization allows are snapshots: a complete save of the current state of a running computer, such as any installed programs or an active running program. The ability to freely and quickly move between snapshots is immensely beneficial as this allows a user to move between different versions of software in only a few minutes instead of waiting for whole disk images to be applied back to the hard drive or having to re-install everything from scratch.

The use of virtualization has greatly improved the process of the internal result validation for the competency test. Not only has using this functionality saved time, but this is all derived from a single feature of virtualization technologies. Decreasing the time spent performing the necessary yet time consuming tasks is something that can benefit any laboratory or practitioner. Virtualization also has many other features that are desirable to the forensic community, such as creating self-contained (air-gapped) investigative virtual machines, and completely standardized hardware that does not change even if you move the virtual machine to different physical computers. These and many other features of virtualization should be sufficient reason to investigate the use of these technologies in any digital forensic environment.

**Virtual System, Tool Testing, Tool Validation**

## B6    Smart Unpacking: Methods for Characterization and Extraction of Embedded Content

*Benjamin Long, BBA\*, NIST, 100 Bureau Drive, Stop 8970, Gaithersburg, MD 20899*

After attending this presentation, attendees will learn about the theoretical and practical frameworks, being developed for the NSRL, to characterize, extract, and measure embedded digital objects using mathematically-based techniques.

This presentation will impact the forensic community by presenting frameworks for addressing the content analysis, data extraction, and measurement of these embedded digital objects.

This work presupposes that digital content can be characterized and classified according to mathematical properties and structures. Discussed is ongoing work as well as how this can form a foundation for validation and measurement of the structures discovered.

This talk describes what Smart Unpacking Research is with respect to the National Software Reference Library (NSRL) project. The NSRL is a project of the National Institute of Standards and Technology (NIST) for collecting and providing identifying information about known files. The NSRL project's goal is to unpack as many files as possible. This research was originally conceived for addressing a particular scenario that occurs frequently in NSRL operations – the need to extract files from compound files (files containing other files) that have no corresponding off-the-shelf unpacker. In addition to this primary scenario, the results of this work may be applied in more general ways. The unpacking methods being developed in this ongoing research are derived by means of modeling the patterns in relevant files using mathematical and other modeling techniques. The objective of this talk is to present the current status of this work and its more general relevance to related problems in the computer forensics domain.

Specifically, this work uses Pattern Theory to develop high quality models for patterns of interest in files, file formats, as well as specific types of content. These models describe how certain patterns are formed and allow us to develop algorithms and techniques for recognizing patterns in certain types of files, file formats, and file content. These algorithms are then implemented using a software framework of parsers to extract files. The parsing framework may also provide measurements to help assess the completeness and quality of such file-extraction operations both for these derived unpackers, as well as, for off-the-shelf unpackers.

Also discusses are how such techniques might be applied to other challenges of general relevance in computer forensics. Generalized versions of this work will be most relevant to one of two tasks: (1) improving understanding of file format and content, as well as, (2) enhanced file carving techniques to extract digital objects out of their digital context.

The focus of the current work is not to reveal the content or structure in encrypted or compressed patterns, but simply to identify data

that might contain embedded compressed or encrypted information. Once identified, such data can be *extracted* as objects for further processing (e.g., decompression or decryption).

**Mathematical Content Analysis, Data Measurement, Content Validation**

## B7      Fixed Size and Variable Size Block Hashes for File Identification

*Douglas R. White, MS\*, 4225 Angell Road, Taneytown, MD 21787-2601*

After attending this presentation, attendees will understand some of the principles of identification of files during investigations of computer systems based on cryptographic hashes of files and partial files.

This presentation will impact the forensic community by introducing the rigor of cryptographic digital file identification at a granular level, which supports statistical identification of objects.

Use of cryptographic hashes or "digital fingerprints" to automatically identify files is absolute when applied to a file as a whole, where the file is unambiguously categorized. When dealing with morphing digital objects, such sorting leaves many files to be dealt with by manual review.

Block hashing is a method of applying the cryptographic algorithms to smaller-than-file size portions of the suspect data. Previous work used cryptographic hashes to "fingerprint" portions of data files, which assist investigators in identification of modified and partially deleted suspect data. Such cryptographic hashes cannot be used to identify similarities between data.

In this case study, cryptographic hashes and "spamsum" fingerprints of corresponding variable sized blocks are computed. The aggregation of the block hash values allow statistical probabilities of identification of suspect files, taking the dynamic nature of digital objects into consideration. The association of a cryptographic hash with spamsum combines positive file identification with a method of identifying similar file content. With this information, investigators can identify portions of uncataloged files which are similar to portions of known, cataloged files.

Examples of practical applications of this technique will be presented. Files from 90 computer systems within one organization were processed. Examples of installation of common software applications can be identified, despite installation modifications. Identification of shared documents can be identified, including edit changes. This work addresses the use of simple anti-forensics methods to defeat automated file identification.

**Cryptographic Hash, File Identification, Block Hash**

## B8      Computer Forensic Tool Testing Strategies

*James R. Lyle, PhD\*, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8970, Gaithersburg, MD 20899*

After attending this presentation, attendees will become aware of some of the strategies used by the Computer Forensics Tool Testing (CFTT) project at the National Institute of Standards and Technology (NIST) for testing computer forensic tools used in the acquisition of digital evidence.

This presentation will impact the forensic community by increasing awareness that the impact tool test strategies have on the ability of tool testing to reveal anomalies in tool behavior.

The Computer Forensics Tool Testing (CFTT) project at the National Institute of Standards and Technology develops methodologies for testing computer forensic tools. The developed test methodologies to several tools in the areas of disk imaging and write blocking have been applied and test strategies for testing storage erasing, deleted file recovery, and string searching are being developed. A test strategy should cover all tool features and also give the tool opportunities to fail in easily detectable ways.

For example, good forensic practice is to start by writing zeros to any pieces of media that would be used in an examination of digital data. However, one common way for a tool to fail is to place information in the wrong location. If a block of zeros is transposed with another block of zeros the switch is undetectable. A better practice for media initialization during testing is to write unique content to each disk sector. This has the advantage that out of place data is easy to recognize. If the unique data also includes the original location of each sector then knowing the original location may be helpful in characterization of the tool behavior.

Disk imaging involves acquiring an image of either a physical hard drive or a disk partition, also called a logical drive. A disk imaging tool functions by reading each sector from the drive to be examined and creating either an image file or a clone of the original on a similar device. An image file contains all of the information to exactly reconstitute the original hard drive. While an image file may be stored as a bit for bit copy of the original, it is usually compressed in some way to save space.

Write Blocking is used to protect original digital data from modification during acquisition or preliminary inspection in order to determine relevance to an investigation.

Storage erasing, as considered by CFTT, is for storage device reuse within an organization rather than for disposal or transfer to a destination outside the organization.

This presentation examines selected test cases and test procedures used by the CFTT project to demonstrate the kinds of tool errors that can be revealed by each strategy.

**Digital, Tool Testing, Software**

## B9      Applying Advanced Search Techniques to Digital Forensics

*Brian D. Carrier, PhD\*, Basis Technology, One Alewife Center, Cambridge, MA 02140*

After attending this presentation, attendees will have a better understanding of what search techniques exist, but are not yet being applied to digital forensics. Attendees will see an example of how these techniques can be applied to digital forensics tools.

This presentation will impact the forensic science community by discussing and talking about how research advances in other fields (namely information retrieval) can be applied to digital forensics to help an investigator more quickly locate evidence.

Keyword searching is common in a digital investigation, but primitive methods are currently being used. Keywords are entered and a list of files with the keyword is given. The files could be listed by file name, by the order the search tool found them in, or something else. It is similar to searching the web 10 years ago. There have been many advances in search techniques that could be applied to digital investigations to help find evidence more quickly. Examples of advances include faceted search, clustering search results by topic, generating automated summaries of documents, and improved ranking. These techniques would allow the investigator to more quickly review search results and ignore the false positives. This presentation will provide an overview of these technologies and demonstrate how they can be used in an investigation.

**Digital Evidence, Search, Analysis Tools**

## B10 An Odyssey Into Lesser Known Regions of Embedded Metadata in Microsoft File Formats

*Eoghan Casey, MA\*, ONKC LLC, 3014 Abell Avenue, Baltimore, MD 21218*

After attending this presentation, attendees will understand how to find previously unknown metadata embedded in digital files that can be critical to an investigation.

This presentation will impact the forensic community by showing forensic examiners, at a practical level, how to uncover lesser-known metadata in digital files. At a higher level, this presentation demonstrates the limitations of file format documentation, existing forensic tools, and the importance of conducting methodical experiments and tests in digital forensics. Furthermore, to bring this process into the realm of science, the methodology used in all three cases is formalized to help forensic examiners repeat the process in other contexts and apply it to other file formats.

A few bytes buried in a digital file can contain crucial details in a case, like remnants of activities that contradict suspect statements, or incriminating text from prior versions of an e-mail. The main challenge for forensic examiners is that the most useful embedded metadata can be buried the deepest. Conversely, the fact that this information is difficult to locate means that it is harder to alter or destroy, and may persist despite the best efforts of the subject in an investigation.

Three cases are presented that made use of lesser known metadata in Microsoft file formats: Word, Excel, and Outlook. The embedded data used in these investigations are poorly documented. Furthermore, forensic tools are ineffective at extracting this information. This presentation guides attendees through an odyssey into Microsoft file formats, using a combination of research and experimentation to uncover important clues embedded (and in one instance encoded) within a file.

**Digital Evidence, Embedded Metadata, Digital File Formats**

## B11 The Impact of Multicore CPUs and Graphics Processing Units (GPUs) on Digital Forensics Tool Design

*Golden G. Richard III, PhD\*, Department of Computer Science, University of New Orleans, New Orleans, LA 70148*

After attending this presentation, attendees will understand the role that multicore CPUs and Graphics Processing Units (GPUs) can play in substantially increasing the performance of tools that process digital evidence. The motivation for "massively threaded" tool designs that support both multicore CPUs and GPUs will be discussed and both the possibilities and limitations of this approach to speeding up digital forensics processing will be covered.

This presentation will impact the forensic community by exposing mechanisms for substantially increasing the performance of digital forensics tools on commodity hardware, with little or no additional hardware expenses, albeit with increased effort on the part of tool developers. This work is important because higher performance tools are critical to deal with the increasing size of investigative targets.

Since the size and complexity of digital forensics targets continues to grow, with commodity disk sizes now exceeding 1TB, it is crucial that tool developers increase the performance of tools that process digital evidence. This is important both to ensure that cases can be processed rapidly to provide timely results and to avoid aggravating the persistent problem of case backlogs. Good tool design plays an important role in rapid evidence processing, but single-threaded designs that process

evidence using only a single CPU cannot be scaled up to deal with ever-increasing target sizes. Therefore, alternative mechanisms must be considered, including more effective use of available computing resources, such as multicore CPUs and high-performance Graphics Processing Units (GPUs). Modern CPUs now commonly use multiple compute cores with lowered clock speeds (e.g., the Intel Core2Duo) in favor of single-core designs with high clock speeds (e.g., the Pentium 4 series and earlier). There has also been a major architectural shift in GPU designs, with modern GPUs providing hundreds of (relatively) general purpose processors instead of very specialized graphics processors. Since most current-generation tools are single threaded, they are generally unable to take advantage of the compute resources offered by multicore CPUs and GPUs. The transition to simple multithreading in tools to fully utilize multicore CPUs is a first (and easier) step in the right direction. But in this presentation it will be argued that new massively threaded digital forensics tool designs are needed and the role that GPUs can play should be carefully considered. Modern GPUs are essentially "supercomputers on a card" and with careful programming can yield very significant performance improvements for a variety of problems. But the associated programming issues are non-trivial and care must be exercised in dividing work between the host CPU and GPUs for maximal performance gains.

Results of some recent efforts to port critical digital forensics operations to GPUs and multicore CPUs to increase tool performance will be presented. The focus will be on file carving, with performance results comparing single-threaded designs, simple multithreading on multicore CPUs, and GPU implementations presented.

**Digital Forensics, Graphics Processing Units, High Performance Computing**

## B12 Supporting Cyber Crime Investigation With the UAB Spam Data Mine

*Chengcui Zhang, PhD\*, CH 127, 1530 3rd Avenue South, Birmingham, AL 35294; Chun Wei, MS, Wei-Bang Chen, MS, Richa Tiwari, MS, and Xin Chen, PhD, CH 128, 1530 3rd Avenue South, Birmingham, AL 35294; and Gary Warner, BS, CH 100, 1530 3rd Avenue South, Birmingham, AL 35294;*

After attending this presentation, attendees will understand how cybercrime investigations can be assisted and how additional evidence of guilt can be gathered through queries to the UAB Spam Data Mine. The UAB Spam Data Mine gathers millions of email messages together into a relational database which supports rich queries as well as complex data analysis to reveal non-intuitive relationships between the cybercrime events to be identified. Online for more than a year, the Data Mine has been successfully used to merge multiple phishing cases against several brands into single cases, and to provide additional data used in the sentencing portions of cases to prove dates and durations of criminal activity in several cases in multiple countries.

The Spam Data Mine will be explained, including the sources for the millions of emails, and the method of parsing, analyzing, and clustering the data. How the Data Mine has been used successfully as the starting point of a successful Malware Investigation will be demonstrated, proving that multiple seemingly unrelated malware attacks were actually a single attack aimed at stealing financial account information through keystroke logging of compromised computers, and leading to identification and arrest of involved perpetrators. In many cases, the Spam Data Mine was able to rapidly conclude that a malware attack was underway, even when the anti-virus products had not yet been updated to provide signatures to detect the emergent malware.

In the second part of this presentation, also discussed is how spam campaigns which use "image-based" spam can be successfully clustered into their appropriate campaigns, even when the images are obscured to

prevent successful optical character recognition (OCR). A unique approach to separating complex images into several layers for deeper analysis and matching will be explained. A case study will be presented where image analysis was able to identify many spam messages all related to the same "Stock Market Pump and Dump" campaigns which will be used to illustrate the possibilities that Image-based clustering of emails can achieve to assist Law Enforcement. The ability to rapidly identify whether a new spam message is part of an existing criminal operation or is something new will be illustrated.

While the computer science aspects of data mining and image processing techniques will be discussed in some detail, many examples of the types of complex law enforcement queries that can be supported by the UAB Spam Data Mine will be provided. Illustrations of appropriate ways to use these new capabilities in support of investigative efforts will be discussed for many scenarios.

**Computer Forensics, Spam, Spam Images**

## B13    Digital Media Players — Recent Research and a Cautionary Tale

*Jessica Reust Smith, MFS\*, Stroz Friedberg, LLC, 1150 Connecticut Avenue Northwest, Suite 200, Washington DC, 20036; Thomas Owley, MD, 1747 West Roosevelt Road, Chicago, IL 60608; Edward Zawadzki, DO, 144 West 12th Street, New York, NY 10021; Soyna Owley, MD, 1500 Waters Place, 10011, Bronx, NY 10461; and Stephen B. Billick, MD, 11 East 68th Street, Suite 1B, New York, NY 10065-4955*

After attending this presentation, attendees will gain knowledge of recent research and analysis performed on digital media players, with a specific emphasis on Apple iPod™ devices and the analysis of metadata for generating timelines and determining user activity.

This presentation will impact the forensic community by serving as a cautionary tale and reminder about the challenges involved when preserving, storing, and analyzing devices as dynamic as digital media players.

Digital media players are fast becoming as ubiquitous as cell phones, and are turning up in increasing numbers in forensic investigations of both civil and criminal matters. A jogger is raped and her digital media player is missing. A suspect is arrested with a digital media player in his possession. What can examination of this device do to help determine the guilt or innocence of the suspect? An employee is accused of industrial espionage. A digital media player is turned over for examination. Could this device have been used in the commission of this crime? What evidence can be extracted from the digital media player to help build a timeline of the commission of the alleged offense?

Digital media players vary greatly both between and within manufacturers. For example, since the introduction of the Apple iPod™, only seven years ago, there have been 15 different hardware versions released. Even within each version there are differences resulting from firmware updates, file system formats, and syncing methods. All of these possible combinations result in unique behaviors that can impact the conclusions that can be drawn from forensic analysis. What happens when the battery dies while it is stored in your evidence room? What are the forensic consequences of playing a song or simply the passage of time? Can you verify the MD5 hash of your forensic duplicate with the original evidence if you allow it to sync with your forensic workstation?

When performing forensic tool/methodology testing and evaluation, your ability to duplicate the hardware, firmware, file system, and syncing environment can significantly impact your results. For this reason, relying on the published results of other forensic researchers without performing sufficient verification may not be wise. When building a timeline of the user activities it is important to have, among other things, an in-depth knowledge of both the file system on which the activity took place and the applications that are involved in the activity.

Metadata that can be used in timeline generation is stored, both on the device and within the software application data store used for syncing (most frequently iTunes™). What are the implications on your forensic examination for a two year old device that has been sync'd with each new version of syncing software as they were released? Can you rely on the metadata for each digital media file to be consistent?

Attendees of this presentation will learn forensic examination techniques for extracting valuable evidence from digital media players, drawn from both applied research and actual investigations. This presentation will also show a forensic examiner what they can do to avoid some of the forensic pitfalls caused by the fast changing digital media player environment.

**iPod, Timeline Generation, Metadata Analysis**

## B14    Using Computational Forensic Linguistics to Screen Pedophilic Communications

*Carole E. Chaski, PhD\*, Institute for Linguistic Evidence, 25100 Trinity Drive, Georgetown, DE 19947-6585; and Raye Croghan, BS, ALIAS Technology, LLC, 25100 Trinity Drive, Georgetown, DE 19947-6585*

After attending this presentation, attendees will understand a validated computational forensic linguistic method for assessing two communication types, threats and pedophilic grooming, in order to screen for pedophilic communications.

This presentation will impact the forensic and legal communities by delivering a method to discover early warning evidence of threatening and grooming behavior that results in child endangerment, abduction, and sexual assault.

Most adults experience threats and pedophilic grooming communications after the fact, when emotional or physical harm has been inflicted. Children, by virtue of their age, are even less likely to be able to accurately evaluate these covert communication types. Lack of exposure to such communications means that most of us are not able to recognize these communication types for what they are, even if we recognize that something is not right in the situation. Yet the ability to recognize and accurately classify different types of problematic texts has obvious survivability, as well as, investigative value in evaluating recidivism potential for convicted and repeat offenders. Computational Forensic Linguistics provides objective, intelligent classification for these rarely-experienced and very problematic text types.

As a branch of natural language engineering, Computational Forensic Linguistics quantifies specific linguistic features in text and dialog, and then subjects this quantification to statistical analysis for classification of documents into forensically-significant categories. ALIAS, Automated Linguistic Identification and Assessment System (Chaski 2005, 2007, 1997) is a computational forensic linguistic program with components for authorship, witness statement relatedness, and other forensically-significant questions. In this presentation, two components of ALIAS, ThreatAssess and PREText are discussed.

ALIAS ThreatAssess provides a very rapid (milliseconds) assessment of a text to determine if it is classified as a real threat or not. Using a database of real threat letters which have been involved in investigation or litigation and the Chaski Writing Sample Database of simulated threat letters, apologies, love letters, complaints, and angry letters as comparison texts, a cross-validated statistical model for classifying texts has been developed. Like the threat text type, each comparison text type has an interpersonal and emotional communicative purpose and therefore represents a good foil. Each new text fed into ThreatAssess is classified as either a real threat or a comparison type based on the statistical model whose accuracy is at least 92% with a maximum of 100%.

Built on ALIAS ThreatAssess, ALIAS PREText, or PREdator Text, provides a very rapid assessment of a text and/or a chat dialog to

determine if it is classified as sexual predatory grooming or not. ALIAS PREText was developed using several different types of pedophilic communications: (1) pedophile to pedophile, (2) pedophile to victim, (3) pro-pedophile activism, (4) risky communications, and (5) defensive pedophile communications. Pedophile to pedophile data includes personal interactions between pedophiles dating back to 1996 as associated with pedophile participation in special interest pro-pedophile only membership groups where electronic communications took place through email, forums, and electronic chat. Pedophile to victim data includes grooming tactics captured between a pedophile and a child or an adult informant posing as a child where the pedophile acted on the chat by appearing physically to meet the minor victim. Many of these chats have been used in court as part of the conviction process. Pro-pedophile activism data includes known pro-pedophile activism web sites, blog articles, papers and letters promoting the pedophilia cause in defense of perceived persecution by society. Risky communication data includes electronic interaction between adults curious about pedophilic tendencies and the normalization of the perversion by the pedophile community and recruitment of new pro-pedophile member tactics. Defensive pedophile communication data includes electronic communications among pedophiles with convictions and/or admitted activities and attraction to minors despite severe penalties, including overt/covert threats against countries and persons illegalizing child pornography and persons engaged in prosecution or anti-pedophile activism.

PREText implements a statistical model of these different communication types, providing a score based on an empirically-derived threshold. ALIAS PREText's objective, quantitative, statistically-validated scoring can be used to develop techniques and training in pedophilic cybercrime investigations, to provide cloaking for investigators, and to present scientific evidence to judges and juries about communications which are, fortunately, unlikely to have been experienced firsthand by the triers of fact.

**Pedophilic Communications, Forensic Linguistics, Predatory Grooming**

## B15 Application of Natural Language Processing to the Digital Forensic Process

*Mark Pollitt, MS\*, University of Central Florida, PO Box 162367, Orlando, FL 32816-2367; and Anne Diekema, PhD, Center for Natural Language Processing, Syracuse University, 335 Hinds Hall, Syracuse, NY 13244*

After attending this presentation, attendees will understand the level structure of natural language processing, the corresponding levels of abstraction and access in digital forensics, and how the two taxonomies are related.

This presentation will impact the forensic community by introducing the digital forensic community to the theories and approaches utilized in the natural language processing community.

Since the second World War, computer scientists and others have struggled to find computational methods to translate, understand, and mimic human communications. What has evolved is an interdisciplinary approach known collectively as natural language processing (NLP). The literature in this community includes the notion of "levels of language" which describe the different ways in which "text" in the broadest sense communicates meaning. These include: phonology, morphology, lexical, syntactic, semantic, discourse, and pragmatic.

The digital forensic community faces a somewhat similar problem in that meaning is stored on computers at a number of different levels. The context and therefore the meaning of any particular data, from a digital forensic perspective, can be altered by the various levels of access/abstraction including the physical media, operating system, file system, application, and content. The metadata from each of these layers provide additional context that shapes the meaning of the data.

This presentation will provide a brief overview of the history of NLP, an explanation of the NLP levels of language, a review of the digital forensic levels of access/abstraction, discuss the similarities of these two processes and map their correspondence with the goal of identifying NLP techniques and methodologies that can be applied to digital forensics.

**Natural Language Processing, Digital Evidence, Digital Forensics**

## B16 Forensic Analysis of Forensic Analysis of Spyware/Monitoring Software

*Don L. Lewis\*, Lakewood Police Department, 445 South Allison Parkway, Lakewood, CO 80226*

After attending this presentation, attendees will be familiar with the challenges presented by the covert nature of spyware/monitoring software. An approach to identify and recover the application and its data files will be presented.

This presentation will impact the forensic community by exploring how the monitoring software, SpectorPro, is designed to be invisible to the computer user in order to avoid detection, but this results in a significant challenge for forensic examination.

Spyware/Monitoring software is marketed to consumers and businesses to monitor activities of children or employees. It is designed to be invisible to the computer user in order to avoid detection, but this results in a significant challenge for forensic examination. This presentation is the result of a case study and research in how to identify and examine spyware/monitoring software.

There are also monitoring emailer applications which monitor and email the user activity to the person monitoring an individual. Emailers have some advantages for the forensic examiner, because they send emails that are easily found in an examination. These emails appear in an unencrypted format and are easily viewed and documented. This presentation only deals with the spyware/monitoring application, which is more difficult to identify, process and examine.

**Spyware, Covert Installation, Monitoring Software**

## B17 Testing of Image Quality of In-Car Video Systems

*Herbert L. Blitzer, MBA\*, and Jerry Jeffers, MS, Institute for Forensic Imaging, 338 South Arlington Avenue, Suite 111, Indianapolis, IN 46219*

After attending this presentation, attendees will gain knowledge on how in-car digital video systems are to be tested in the future and how this might affect forensic video analyses.

This presentation will impact the forensic community by showing that the use of in-car digital video is expected to continue growing and that the IACP has promulgated requirements that departments can utilize in the acquisition of systems. The result is expected to be an increase in the quality and some degree of predictability of performance of these systems. This will have an impact on how forensic video analysts interpret their findings in the future. It will help anticipate what sized objects might be reproduced, what colors might be reliable, and how movements can be interpreted.

The use of in-car video systems in police and other emergency vehicles is growing rapidly. Unfortunately, there are many aspects that are important to a successful system and guidelines for these systems are just emerging. Some of these aspects deal with: physical properties, electrical properties, system integration properties, and image quality.

Setting requirements for image quality is very difficult and a set of properties has been selected for use at this time. This paper will deal with those properties, how they are measured, and what performance details they cover.

Over the past few years, a team lead by the International Association of Chiefs of Police (IACP) has been working to determine the physical layouts that are involved in in-car video recording and they have set some basic indications of the types of objects that should be resolvable. They have measured the lighting conditions that might be encountered and the colors that might appear in scenes. They also have indications of the movements of interest in a typical scenario. The testing routines are based on these findings.

The properties measured are static resolution, dynamic modulation, dynamic range, aspect ratio, and color fidelity. Static resolution is measured both vertically and horizontally using targets that are consistent with the objects of interest at a typical scene. Bar charts are used and the test is designed to show if the system can reproduce a certain standard or not. It is a pass fail test and not an engineering measurement. Dynamic modulation started out as a test of resolution of a moving target, but testing has shown that what is really being measured is the degree of modulation an image maintains as the target moves faster and faster. This turns out to depend on the sensitivity of the light levels, sensor and the shutter speeds of the camera. Hence, this property is now referred to as modulation of a dynamic target. The system's compression routines can have a significant impact on these results. Dynamic range is measure using a sensitometer with a 10,000:1 test target. The system's monotonic response above noise threshold point and below the saturation point is examined. Most of the cameras were in the range of about 100:1, which is a bit short of the range found in a number of typical scenes. Comparisons to digital still cameras are shown for context. To measure color fidelity, a Macbeth Corporation Color Checker is used along with 5,000° Kelvin lamps. Frames are then taken from the video as an officer would when analyzing a recording is sampled. The CIE/Lab values for the primary colors and gray level patches are measured and individually compared to the correct values for those patches. Then a figure of merit is calculated base on a mean square error calculation.

The result of measuring a number of cameras, each with its respective software, is shown. As a general rule, the analog cameras are better at dynamic modulation, but worse in the other respects. The high definition cameras are very good at color fidelity and static resolution, but sensitive to light levels when examining dynamic modulation. Dynamic range measurements are comparable across all the cameras tested, and all are marginal relative to the application requirement. All are low relative to the range that can be achieved with digital still cameras.

The testing described in this paper is the basis for the image quality portions of the current IACP, in-car digital video specification. These may change as new technology is developed and as practical experience under the current regime is recorded. For example, there was discussion of moving color test targets, but this in not measured in the current specification.

**In-Car Video, Forensic Video, Testing**

## B18 A Subjective Video Quality Test Method for the Assessment of Recorded Surveillance Video

*Mark A. McFarland, ME*, U.S. Department of Commerce, NTIA/ITST, 325 Broadway, Boulder, CO 80305*

After attending this presentation, attendees will learn of a new test method developed by the National Telecommunications and Information Administration (NTIA) that is suitable for assessing the subjective video quality of surveillance (and other task-based) video.

Attendees will also understand the unique problems associated with assessing the quality of surveillance video and why extant testing recommendations on subjective video quality assessment (developed by the International Telecommunication Union (ITU)), cannot be applied to the surveillance video testing.

This presentation will impact the forensic science community by giving the forensic video community a standardized method to assess the quality of surveillance video. Law enforcement organizations have noted problems arising from low quality surveillance video and have been developing guidelines that aim to improve the quality of surveillance video; yet no method exists that can be used to measure this quality. The test method described in this presentation provides a new testing method, which may be used to measure the subjective video quality of surveillance (and other task-based) video.

The quality of surveillance video impacts our law enforcement communities, courts, and the public. Poor surveillance video quality could result in critical evidence being dismissed and criminals remaining at large or being set free.

The quality of surveillance video is of major importance to the law enforcement community. Quality is defined as the minimum acceptable levels of impairments that make it possible for law enforcement to utilize the recorded surveillance video to do its job and identify images in the video that are pertinent to an investigation and use those images to help identify, apprehend, and prosecute criminals. Once a crime is committed, the surveillance video may become critical evidence for the purpose of identifying what happened and who and what was involved in the crime. The surveillance video helps law enforcement piece together the events, objects, and individuals related to the crime and apprehend and prosecute the suspect(s). The surveillance video is essential evidence in many criminal cases.

Low quality surveillance video is a problem for the law enforcement community because it impedes its ability to do its job. Low quality recordings do not give law enforcement the level of detail needed to identify a suspect or an object or to piece together the events of a crime. Many groups have been looking at improving this quality. The National Telecommunications and Information Administration's (NTIA) Institute for Telecommunications Sciences (ITS) has undertaken research in this area. Part of this research resulted in the development of a subjective video quality test method suitable to measure a video analyst's assessment of that quality.

Methods for subjective video quality testing have been proposed by the International Telecommunication Union (ITU), the Motion Pictures Expert Group (MPEG), military image quality researchers, and others over the previous decades. The method described in this abstract synthesizes recommendations of these groups, along with recommendations by law enforcement video analysts, and proposes a new test method which enables the subjective quality of surveillance video to be measured in a standardized manner.

The unique problems associated with assessing the quality of surveillance video are presented. Reasons why extant testing recommendations on subjective video quality assessment (developed by the ITU) cannot be applied to the surveillance video testing are also discussed.

This test method will benefit the law enforcement community and video quality researchers because it provides a standardized method to assess the effectiveness of guidelines and recommendations which were developed to improve the quality of surveillance video. This method has been presented to the International Telecommunication Union (ITU), and is currently a draft recommendation by the ITU's Study Group 9.

**Surveillance Video Quality, Subjective Video Quality, Video Quality Assessment**

## B19 Examining Photo Response Non-Uniformity for the Comparison of Cameras

*Zeno J. Geradts, PhD\*, Maarten van der Mark, BS, and Wiger Van Houten, MS, Netherlands Forensic Institute, Ministry of Justice, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS*

After attending this presentation, attendees will learn what conclusions can be drawn from Photo Response Non-Uniformity, how to validate the method, and methods for examination and practical software.

This presentation will impact the forensic community by providing practical methods of validation and some statistical background for determining if images are made with the same camera.

Camera identification, based on pixel artifacts, has been widely known in forensic science for over a decade. Currently, sensors (CMOS/CCD) are manufactured with no pixel defects, and the Photo Response Non-Uniformity (PRNU) can be used as a comparison measure for a specific camera. The PRNU is a measure to identify cameras based on the slight variations between pixels which is characteristic for a camera and claimed not to vary in time.

For practical use in forensic science, it is important to validate the results and also the causes of the PRNU. This paper aims to answer two questions:

- Is there a practical method for measuring PRNU?
- What are the causes of PRNU and statistics behind it?

In the past a Matlab-script for reading the PRNU was developed for low resolution cameras. Since it is not easy to use on a wide scale, it was converted to Java coupled with a database of cameras. The goal of this application is to help forensic researchers and others to determine the source of a digital photographic image. To achieve this goal both the digital image(s) and the suspected source camera is needed. It is also necessary to have several other digital cameras available, preferably of the same brand and model to compare the results. This application works by extracting an average Photo Response Non-Uniformity pattern, a form of chip specific noise, from the images of interest. The correlation between the PRNU and reference patterns from several cameras is calculated. The reference pattern that has the highest correlation is most likely to be the source camera for the image of interest.

The following steps are taken during the extraction process of the PRNU:

- Blocks of pixels are averaged to reduce jpeg artifacts.
- A convolution with a small Gaussian filter is performed.
- The filtered image is subtracted from the original to get the filtered noise.
- The image edges are set to zero (convolution causes errors near the edges).
- Multiple PRNU patterns are averaged to one pattern.
- The PRNU patterns can be stored in a database and a hit list will appear with a ranking. When using this program it is important to validate the results by using several same type cameras to know how random the pattern is. The software for this database is named PRNU Compare and available from www.sourceforge.net.

Within this research an attempt is made to find a statistical measure to objectively qualify the value of the evidence, by dividing the probability density functions under two hypothesis. Based on the correlation found between the PRNU pattern extracted from the questioned image and the flat fields from the suspect's camera a Bayesian conclusion could be drawn. The results are convincing, since the correlation between two images having the same origin is much higher than when this is not the case. Due to the large amounts of test data needed to reliably estimate the density functions, it is not a practical approach. A few alternative approaches are mentioned, which may be useful for continued research on solving this issue.

Different methods for concluding the results are discussed as well as future research within the European Network of Excellence FIDIS (www.fidis.net), where an attempt is made to link cameras based on PRNU, (e.g., You Tube).

**PRNU, Likelihood Ratio, Camera Identification**

## B20 Determination of Time of Recording With Electric Network Frequency (ENF)

*Maarten Huijbrechtse, BS, and Zeno J. Geradts, PhD\*, Netherlands Forensic Institute, Ministry of Justice, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS*

After attending this presentation, attendees will gain knowledge of how Electric Network Frequency assists in determining the authenticity of a recording, tampering, the time of recording utilizing ENF, how to collect ENF, and validation of results with statistical background.

This presentation will impact the forensic community by giving an overview of the current status of ENF-research, tests to validate the results, and to use a Bayesian approach for conclusions.

In casework, sometimes there are doubts regarding the authenticity of a recording. Has a crime been committed at a certain time, when for example someone recorded it with a video camera on a phone? There can be time stamps on the recording; however, sometimes there is also the signal of the electricity network available on the recording.

The European network has 50 Hz as mail frequency, whereas the U.S. network has 60 Hz. However, it is known from various research studies, for example by Grigoras, that over time, the frequency is not constant, but fluctuates around 50 Hz in a presumably random way. At each point in time, the fluctuation is the same throughout the entire network.

It is also known that a digital audio recording can contain the ENF signal if it has been recorded with mains powered equipment (Grigoras). Further, according to Kajstura et al., it is possible to detect the ENF signal in a recording made with battery-powered equipment.

Grigoras and Kajstura et al. have shown that it is possible to verify or falsify a questioned time of recording by comparing the ENF signal from the recording with a database of the ENF fluctuation. The natural follow-up question is: Can we use the ENF signal from a digital audio recording to determine its (unknown) time of recording? Our research aims at answering this question.

A database was created of the ENF fluctuation that was recorded from September 2005 to February, 2007 (with some interruptions). This database is reported to differ less than 2 mHz from frequency measurements by the Swiss ETRANS company.

This database is used to test the randomness of the ENF fluctuation. This was completed by computing typical correlation coefficients ($r$) and root-mean-squared differences ($e$) for two separate pieces of equal length from the database. With $r$ close to 1 and $e$ close to 0, the pieces are determined to be (almost) identical. Ideally, this only happens when the two pieces are in fact not separate ones, but the same ones.

Furthermore, ENF fluctuation were collected for a month. During this collection process, several audio recordings were made both in uncompressed (.WAV) and compressed (.MP3) format. By matching the ENF signal from these recordings with the collected ENF fluctuation, testing to determine whether $r$ and $e$ are significantly closer to 1 and 0 respectively than the typical values found from the database was conducted, and thus whether a recording can be uniquely positioned in time.

Future research could be aimed at determining with which network a certain recording has been made. The difference for example between the American network (60 Hz) and the European network (50 Hz) might be obvious. A recording can also be made with a generator, which could also have certain ENF patterns. Future research within this field could

also include checking for patterns derived from ENF for example in the images of video streams or other sources.

For forensic research it would be necessary to have ENF databases from electricity networks that are not connected and accessible to other forensic scientists. A java applet for acquisition has been developed for the acquisition. A challenge is to have a reliable signal from the different networks in the world, in which different laboratories in the world can acquire data from the different networks. When large ENF databases from different networks are available, it is possible to compare the databases, which helps in determining authenticity of a recording in forensic science. Also in the forensic conclusion of the report it becomes possible to conclude in a Bayesian approach, since statistics are available from these database, and conclusions drawn are more objective.

**ENF, Electric Network Frequency, Audio**

estimation of the error made in the speed calculation is directly performed. Validation recordings from the same type of car, traveling with known speed along the path that was traveled by the car in the questioned video were made. For this, the same recording equipment was used as for the questioned video. These validation recordings of the car were made at different speeds, chosen around the estimated speed of the car in the questioned video. From the validation recordings the speed of the car was derived in the same way as for the questioned video. The difference between the calculated speed and the known speed was used to calculate the variation around the average difference. This variation was used to estimate a 95% confidence interval for the calculated questioned speed.

**CCTV, Photogrammetry, Statistics**

# B21    Car Speed From CCTV Images

*Bart Hoogeboom, MS\*, and Ivo Alberink, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS*

After attending this presentation, attendees will understand some principles of calculating the speed of a car from CCTV images and how to gain insight in the measurement errors.

This presentation will impact the forensic community by providing a better understanding of measurement errors in speed calculations from CCTV footage.

In forensic investigation, on a regular basis the question arises whether the speed of a car shown in a video can be determined. The video is usually obtained from a Closed Circuit Television (CCTV) system containing time lapse, black & white, or color recording.

The speed of the questioned car is calculated by measuring the path of the car between two images and calculating the time difference between the two images given the time by the CCTV system. To measure the path of the car between two images a three dimensional computer model from the road and characteristic points may be used. The model can be looked upon from the same perspective as the questioned images, and with the computer model projected on the questioned images the position of the car can be determined. As for every measurement in real life, there will be a difference between measured and real path, so measured paths, and time intervals accordingly, are always estimates for actual values. In this case study, two different situations are discussed: in the first case a car was recorded by two different cameras from the same CCTV system; in the second case a car was recorded by just one camera from a CCTV system. Those two different situations have been approached with different methods for gaining insight into the estimation errors.

In the first method, referred to as the stationary method, the errors made in the path and the time estimation are separated. To measure the path between two images from the two different cameras, a similar car was positioned at the scene of crime, at the position as can be seen in the questioned images. For this, the cameras that took the original questioned video footage were repositioned using the questioned images. For both cameras, the positioning of the car was repeated by different operators, thus producing a variation around an average position per image. The different positions were measured using a land surveying device, and from the resulting drawing the paths between the positions were calculated. For the timing two clocks were started and recorded by the first camera. After this, without stopping the clocks, one clock was moved to the second camera and recorded. The recorded videos of the clocks were used to observe the difference in given time intervals by the CCTV system and the clocks. The variation in the observed errors of these time intervals and their paths were used to estimate a confidence interval for the calculated speed.

In the second method, referred to as the dynamic method, the

# B22    3-Dimensional Analysis of Video Footage

*Gerda Edelman, and Jurrien Bijhold, PhD\*, Netherlands Forensic Institute, Laan van Ypenburg 6, 2497 GB, Den Haag, 2497GB, NETHERLANDS*

After attending this presentation, attendees will be familar with methods for 3-D visualization based on video footage.

This presentation will impact the forensic community by giving new insights on 3-D visualization based on video.

Video footage from CCTV, phonecams, etc. can be used to track, trace, and identify perpetrators. However, with the growing number of video recording devices, the amount of information increases rapidly. This makes it necessary to improve the process of capturing, converting, synchronizing, viewing, and analyzing video files. Surveillance images could be used more effectively with the help of 3-dimensional models of the scenes that are visible in the surveillance images.

First, virtual camera views in 3-D models can help to design a camera plan with an optimal coverage of the areas under surveillance. When these virtual camera views are matched with the real camera views, it becomes possible to estimate the position and speed of people and cars that are visible in the real video images.

With such information, it can be predicted when a person or car might show up in another camera. At the Netherlands Forensic Institute a project is being carried out to reconstruct all movements of people and cars before, during, and after a big incident from analysis of all available video footage.

In this presentation, a brief description of the project is given. Forensic aspects of the interpretation of video footage are demonstrated with video footage from a police investigation and a 3-D model of an urban area. The models are used as a tool for documenting observations in the video, combining these observations with other information sources, and for testing and documenting hypotheses on relations between events in different cameras.

**3-D, CCTV, Video Footage**

# B23    Quantifying Measurement Variation and Evidential Value When Performing Body Height Estimations in Digital Images

*Ivo Alberink, PhD\*, Bart Hoogeboom, MS, and Annabel Bolck, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS*

After attending this presentation, attendees will understand principles of how to cope with measurement errors when performing

body height measurements in images and how to quantify eventual evidential value.

In forensic practice, height estimations on perpetrators visible in video footage from surveillance cameras are regularly requested. The approach to this at the Netherlands Forensic Institute is the following: the crime scene is visited with a number of test persons. A Closed Circuit Television (CCTV) or camera image is selected in which the perpetrator is standing more or less in upright position. The test persons are positioned at the same location and in front of the same camera as the perpetrator on the original footage in as much as possible the same pose. This procedure is called a reconstruction and yields validation readings that allow to correct interpretation of height estimates of the perpetrator.

On the basis of 2-D photographs and fixed location points, a 3-D model of the scene of the crime is created. Using common points of the 3-D model and the camera view on the questioned image, the location and orientation of the camera is determined, and the 3-D model is projected such that it has the same perspective as the camera images. Next, investigators perform height measurements on the test persons and the perpetrator by placing cylinders over the bodies in the 3-D model, from feet to head. The height of the cylinders approximates the actual height of the test persons and perpetrator, reduced by the loss in height by the pose of the perpetrator. Variation between actual and measured heights of the test persons and the perpetrator is introduced by factors like creation of the 3-D model, finding of camera orientation and focal length, presence of lens distortion, pose of the perpetrator in the chosen image, presence and height of head- and footwear, interpretation of head and feet in the images by investigators. This variation may be decomposed into a systematic and a random part. By measuring reference objects in the image, like measuring sticks, an estimate of the systematic error by variation in the modeling of the crime scene can be made. Systematic error by varying height loss because of pose cannot be estimated directly. In practice (casework), systematic errors amount to several centimeters and vary from case to case. Since variation introduced by head- and footwear cannot be removed without extra knowledge, height measurements are usually of the test persons and the perpetrator including head and footwear.

The goal is to answer the following two questions:

1. On the basis of the measurements, how can probability statements be given (confidence intervals) on the actual height of the perpetrator?
2. In case there is a suspect: what is the evidential value, in terms of a Likelihood Ratio, of eventual resemblance of suspect's and perpetrator's height?

These questions have not received much attention in the literature, which has focused more on technical methods than validation. Using normal approximations and the observed variation on test persons, a method is described for obtaining confidence intervals for the height, including head- and footwear, of the perpetrator. Since the number of test persons is usually limited, the result is in terms of the Student-t distribution. In addition, an expression is obtained for the Likelihood Ratio, measuring the strength of evidence of resemblance of the actual height of a suspect and the measured height of the perpetrator. This depends both on the rarity of the estimated perpetrator's height and on its closeness to the suspect's height. The analysis of validation measurements described in the current paper does not depend on the method used and holds up as well if measurements are made on the basis of projective geometry (vanishing points).

**Evidential Value, Body Height Estimation, Confidence intervals**

## B24    Face Recognition on CCTV Materia Using a Biometric System:  Limitations and Opportunities

*Arnout C. Ruifrok, PhD\*, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS*

After attending this presentation, attendees will have a better insight in the limitations and possibilities of automated biometric systems for facial identification using CCTV material.

This presentation will impact the forensic community by qualifying some of the claims made by facial recognition systems.

Biometric face recognition is still advocated as a good option for person identification and detection of people on watch lists. However, the current state of the art in face recognition is mostly not sufficient for forensic applications. Although some of the techniques reach reasonably high levels of recognition under controlled circumstances with frontal face images, of course, surveillance images hardly ever capture a suspect frontal face, with good lighting conditions, and a neutral facial expression. Also sharpness and resolution are, in general, far from optimal. Of interest to the forensic use of biometric systems is knowledge about the reliability of the matching results, even under imperfect conditions.

The performance of face recognition software was studied using surveillance images from six different analog cameras and camera-positions. The surveillance material was recorded at 4CIF (704x576 pixels) at 12 frames per second. Volunteers were asked to walk along a predefined path and stop walking and look left and right at 4 positions. The frontal-most images at each position were selected for the analysis. Verification match results were used to construct receiver-operator curves (ROC), and the Equal Error Rate (EER), error rate at the setting resulting in equal rates of false accepts and false rejects was used as performance criteria.

When using good quality controlled lighting and frontal pose images, an EER of 1.5% can be reached using automated face recognition software. However, when using passport-type but less controlled entrance-card images, the EER increased to 9%. Even with cameras at eye-height and fully zoomed-in the EER increased to 24-30% at distances of 1.5-3.5 m. When the subjects were wearing a baseball cap, EER increased 4-10% compared to bare-headed images. Images from a teller-machine like camera position performed relatively well. These images resulted in an EER of 16% with people looking into the camera, but performance dropped to an EER of 37% when people looked straight ahead, when a database of high quality controlled frontal images was used. However, when a database with images from a similar low position camera was used, the EER improved to 19% for the teller-machine images with people looking straight ahead and to 9% when people looked straight into the camera. At almost all camera positions the use of a reference database with images from the same camera position outperformed the use of full frontal images as reference database. This indicates that full frontal images are not always the best reference set for automated face recognition. Preferably images from the same camera and position should be used.

The absolute match-values generated by the recognition software should be viewed with care, as low quality images compared to a low quality database resulted in high match-values for matching as well as mismatching images, with high EER values as consequence. The data even suggests that the mismatch-values of an image with a database of images of a similar quality may be predictive of the EER of the system. This means that the evidential value of an image may be predicted by the mismatch value with images of similar quality, providing the opportunity to establish the evidential value of the CCTV image without suspect information.

**Facial Recognition, CCTV Images, Biometrics**

## B25 A Morphological Classification of Eyebrows to Aid in Forensic Facial Comparisons

*Matthew E. Graves, MFS\*, Carl R. Kriigel, BS, and Cristy B. Pruitt, MFS, U.S. Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297*

After attending this presentation, attendees will learn about a facial identification study that supports photographic comparisons as a part of image analysis. In facial identification research, there is very little information pertaining to the uniqueness of eyebrow features to be used in support of facial identification. The basis for conclusions reached through photographic comparison lies in the detection of correspondence or discordance of subject features. At the end of the presentation, the attendees will understand how eyebrow features can be used to support forensic facial comparisons and image analysis examinations. After attending this presentation, attendees will also understand the three main methodologies for forensic facial comparisons. Eyebrow classification based on morphological characteristics for facial comparisons will also be discussed. In addition, the various aspects of the eyebrow that were visualized to create a classification system will be presented along with the results of classifying 112 sets of eyebrows. Finally, further research possibilities in this area will be suggested.

This presentation will impact the forensic community by providing a methodology for the examination of eyebrows when performing forensic facial comparisons or identifications.

The goal of this study was to photograph approximately 100 individuals and then perform side-by-side photographic comparisons to determine if the eyebrow contained individual characteristics to suggest they are unique. In order to evaluate the uniqueness of eyebrows and the morphological characteristics they possess, it was necessary to develop a database of facial images for examination. For this project, 112 individuals volunteered to be photographed. Each photograph was categorized according to general shape, arch height and size (the width of the eyebrow in this instance). While grouping the images, the number of subsets containing distinct characteristics made it rare if any two eyebrows fell into the same category, and many other features were observed that could prove beneficial in determining eyebrow uniqueness. Therefore, the various characteristics were arranged into a table listing each category and an eyebrow classification. The observable features were then assigned a numerical value within each group.

After evaluating each of the 112 photographs and a total of 224 eyebrows, a spreadsheet containing the classification of each image was compiled. Based on the "Eyebrow Classification Table" developed, there were a total of 17 areas examined to aid in classifying each individual's eyebrows. The spreadsheet depicts that from the 112 individuals who were studied in this project, no two sets of eyebrows classified the same way. Upon analyzing the results obtained from this study, eyebrows appear to be an area that, when adequately examined, may prove valuable as a piece of the puzzle for facial identifications. Since no two individuals' eyebrows were found to be similar in this study, the utilization of a classification system should aid examiners in developing a universal terminology and methodology for the evaluation of eyebrows for individualization.

While all of the classifications of the eyebrows of 112 individuals in this study were found to be unique, there is still much research that must be completed in order to more thoroughly evaluate this method for use as a facial comparison tool. Future research on this topic may include studies on intra-assessor agreement, photographic variables, the effects of facial expressions on eyebrow characteristics, changes in eyebrows over time, and numerous other possibilities.

Overall, the eyebrow area appears to be a region of the face that is distinguishable between individuals. While eyebrow identification cannot and should not be used as a stand-alone source of individualization, when used in conjunction with other methods of facial comparison, it could prove to be extremely useful. The classification system developed may prove beneficial as a starting point for an examiner attempting to identify an individual from a photograph. In the future, it may be possible to create charts and overlays of standards depicting what constitutes various shapes and sizes of eyebrows. Through training, experience, and with the help of exemplar images depicting various characteristics, examiners throughout the forensic science community will become more accustomed to the examination of eyebrows when performing forensic facial comparisons.

**Morphology, Classification, Facial Identification**

## B26 Future Tools for Forensic Digital Audio Analysis

*Kenneth Marr, MS\*, David J. Snyder III, BS\*, and William L. Hinton, BS\*, Federal Bureau of Investigation - FAVIAU, ERF - Building 27958A, Quantico, VA 22135*

After attending this presentation, attendees will understand the changing nature of forensic audio tools and analysis. In addition, attendees will realize that Digital and Multimedia sub-disciplines will have an increasing share of common training, forensic tools, and examination requirements.

This presentation will impact the forensic community, especially the sub-disciplines of computer forensics, forensic audio, video, and image analysis, by showing that interdisciplinary communication and cooperation concerning training and forensic tools are vital and will significantly improve future examinations.

The toolbox for the forensic audio examiner of the future will contain an increasing number of computer forensic methods. A timeline is presented which details the accelerating pace of forensic audio technology and tool development. Current practitioners will recognize many tools and methods which, although important and considered cutting-edge when first developed, have been shelved for more accurate and applicable tools of today. These historical changes highlight the trend that new tools require not only additional qualifications and certifications but also far more technical training for audio examiners. Computer hardware and software analysis, and automated technology will demand higher levels of education and technical degrees in order to explain examination results and implications of the findings to a jury in the courtroom. Issues of tool validation, calibration, and technical applicability are new hurdles that will become more prevalent and necessary when examination results are presented to more and more sophisticated courtroom judges and juries.

Training, qualifications, and laboratory accreditation will become more and more aligned with the field of computer forensics. With the incorporation of forensic audio as part of the Digital and Multimedia Sciences discipline, it is clear that a significant amount of computer forensics training is also needed for many aspects of forensic audio analysis. Only after a significant level of computer-related topics will the training diverge into separate "tracks" for the sub-disciplines of forensic audio, video, and image analysis.

Laboratory accreditation will become more prevalent and expected for both large and small forensic laboratories. This trend may cause a number of smaller facilities to stop forensic audio examinations due to the overhead costs of quality assurance programs, accreditation documentation (e.g., SOP's), ongoing technical requirements, and maintenance costs of both hardware and software tools.

Enhancement of audio recordings now utilizes some powerful new techniques and methods which were not available in the past. New data processing techniques allow an examiner to view an entire audio file visually to identify areas of clipping or distortion, silence, abrupt changes in environment, and background noise levels, etc. The examiner

can then select and apply filtering techniques to the recording in a batch process which usually takes far less time than traditional aural review and filtering in real time. The future holds the likelihood that more sophisticated audio enhancement tools will allow examiners to be even more effective in less time than at any point in the past.

Nowhere has the shift in expectations involved in forensic audio been more profound than in audio authenticity examinations. Many traditional techniques are simply not applicable to today's digital audio authenticity issues. These include magnetic development and physical inspection of analog evidence tapes. Newly designed methods to authenticate recorded files and identify any alterations have been implemented for some law enforcement digital recorders.

Other aspects of forensic audio analysis will also require specialized examination tools. These tools will apply to many aspects of forensic audio including enhancement, authenticity, voice comparison, automated gunshot detection and analysis, and possibly web-based voice surfing capabilities.

It is proposed that the development of any forensic analysis tool of the future must incorporate independent testing, validation, and certification of those tools. The forensic tool validation process must be timely and applicable. Some larger accredited laboratories already have established testing and validation procedures for new tools. Some of the necessary steps in this process include: (1) identification of potential new forensic tools, (2) researching the capabilities and credibility of the manufacturers or source of the new tools, (3) testing the functions and features of the new tools, comparing results to previous tools and to other standard audio discipline techniques, (4) validation of the new tool's functions by an objective testing facility, and (5) certification and documentation of the new tool for use in forensic audio analysis.

An example for a digital audio authentication method designed for certain law enforcement recorders that incorporates several computer forensic tools will be presented.

**Audio, Authenticity, Future Tools**

# B27    The Virtual Digital Forensics Laboratory

*Mark R. McCoy, EdD\*, University of Central Oklahoma, Forensic Science Institute, 100 North University, Edmond, OK 73034*

After attending this presentation, attendees will understand the concept of the Virtual Digital Forensics Laboratory (VDFL), the technology solutions it employs, and the flexibility it provides for digital forensic investigators.

This presentation will impact the forensic science community by proposing an innovative concept to expand the capabilities of digital forensic examiners to examine digital evidence and distribute the results of those examinations.

Law enforcement investigators have attempted to respond to the growing and complex need to investigate all matter of computer related incidents by using stand-alone forensic workstations and limited storage solutions. Digital forensic examiners often find that their cases are held up by cumbersome and inflexible technology that limits their effectiveness. The need to store and examine large quantities of data and the need to provide easy access to examination results to investigators in remote locations has changed the face of the digital forensics laboratory.

A Virtual Computer Forensics Lab (VCFL) is a fairly new concept that applies existing enterprise virtualization technology to current forensic investigative methods. Virtualization technology was introduced in the 1960s to allow the full use of mainframe hardware, but more recently virtualized network, storage, and workstation technologies have matured to the point where they can be used to effectively overcome computer forensics lab constraints. Today virtualization is helping many Information Technology (IT) organizations solve problems with scalability, security, and management. Virtualization can help computer forensic labs do the same.

A computer forensics lab must be able to keep pace with the technology it analyzes, and it must allow investigators secure remote access to forensic tools. Virtualized hosts and virtualized storage, along with strong network encryption, allow organizations the flexibility for multiple investigators to collaborate using the same evidence, while using as many virtual forensic workstations as needed, with a storage system that can scale to hundreds of terabytes.

Virtualization technology is the abstract layer that resides between what is presented and the physical hardware. There are three core virtualized technologies needed to create a virtual lab environment: virtual private networks, virtual machines, and virtualized storage. A fourth (non-virtualized) component, two-factor identity management technologies, is also needed to create a secure and confidential lab environment. This technology can be applied to existing computer forensic labs to create a complete virtualized layer that still meets rigid ASCLD (American Society of Crime Laboratory Directors) requirements.

**Digital Forensics, Virtual Digital Forensics, Virtual Lab**

# B28    The Persistence of Image Files on Digital Camera Memory Cards

*Brian J. Gestring, MS\*, Cedar Crest College, 100 College Drive, Forensic Science Program, Allentown, PA 18104*

After attending this presentation, attendees will learn that it is possible to recover image files from digital camera memory cards after they have been erased and the card reformatted. A number of experiments will be described that illustrate how easy it is to recover these images with commercially available software. Mechanisms will also be discussed which prevent image recovery.

This presentation will impact the forensic community by demonstrating how standard operating procedures (SOPs) are essential for implementing a successful digital imaging platform. This presentation will alert users to the persistence of image files on digital camera memory cards and demonstrate how to effectively clear these cards. This information should be incorporated into any agencies SOP.

The dramatic improvement in digital imaging technology has lead to growing acceptance of digital photography by both the law enforcement and forensic community. As the cost of a good single lens reflex (SLR) style camera continues to drop and the image quality for these digital SLRs (dSLRs) continues to increase, many agencies have elected to replace their traditional film applications with digital photography.

One fundamental difference encountered when switching from film to a digital platform involves the mechanism in which the images are initially recorded. With traditional film, the image is recorded as either a negative or positive (e.g., slide film) image. Since both of these are tangible, it is easier to physically and mentally keep track of them. With digital photography, images are recorded onto memory storage devices in the camera. Once these images are transferred from the card, the cards are cleared and reused again. Often users will utilize the "delete all" or "format card" feature on the camera to clear the cards in between uses. After either of these processes, no images will be visible on the card with either the camera or an external device. Unfortunately, this does not mean that the images are permanently deleted. There are a number of commercially available softwares that are very good at recovering image files from digital camera memory cards. Since the security of images taken for forensic science or other law enforcement applications is paramount, it is important to realize that even "empty" cards may contain images.

To evaluate the parameters where image files can be recovered, experiments were devised using a Nikon D200 dSLR with a 60 mm Micro Nikor lens and a new 2 GB 133 speed CompactFlash™ card. The

camera was set to ISO 100, auto white balance, and the quality was set to Raw + JPEG (large).   Exposure was set manually based upon the TTL lens meter and was set to $f$ 3.2 and 1/60 for all the exposures. PowerPoint was used to make slides with the experiment title, name, and sequential numbering.  Each slide was then photographed with the D200 mounted on a tripod.

For the first experiment 99 images were recorded to fill the card. The images were saved onto an external hard drive and then the "delete all" feature on the camera was used to clear the card.  The card appeared blank when evaluated through the camera or a computer. Commercially available image recovery software was then used to analyze the deleted card and recover any possible images.  All of the RAW and JPEG images were recovered with no loss in resolution.

Without the aid of the image recovery software, the "deleted card" still appeared blank.  The card was then reformatted on the same digital camera.  Again, the card appeared blank to the camera or a computer. Using the image recovery software, all of the RAW and JPEG images were recovered from the deleted and now reformatted card with no loss in resolution.  This same card was then reformatted an additional six times.  After each reformatting, all of the original images were recovered with no loss in resolution.

In the second experiment 51 new images were recorded onto the deleted and reformatted card.  The original images were stored onto an external hard drive and then the images were cleared from the camera using the "delete all" feature.  All of the images from experiment 2 (RAW & JPEG) were recovered with the image recovery software as well as the last 46 RAW and JPEG images from experiment 1.  There was no loss of resolution in any of the images.

A number of additional experiments were performed that fully evaluated the ability of the image recovery software to recover images off of cards thought to be empty.  For those familiar with computers, it might not be surprising that deleted files can be recovered.  Since the outward appearance of digital cameras still resembles that of their film ancestors, the fact that digital cameras are actually computers is often overlooked.  Experiments such as these are necessary to define best practices and create standard operating procedures for digital photography.

**Digital Photography, Memory Cards, Image Recovery**

## C1 Forensic Linguistics: Curious and Instructive Parallels Between Voiceprints and Forensic Stylistics

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After attending this presentation, attendees will be able to recognize the difference between current computational research in speaker and author identification and popular but misguided attempts, á la CSI effect, at using language data as forensic evidence.

This presentation will impact the forensic and legal communities by providing lessons learned from emerging technologies in novel aspects of forensic identification, showing that when invalid methods are held to legal requirements for scientific evidence such methods can at least be hampered if not totally excluded from the court system.

Forensic linguistics provides linguistic analysis as evidence. In forensic linguistics, the linguist focuses on answering forensically-significant questions which have arisen in a criminal or civil case. The two most common questions are: Who is speaking on this tape? and who wrote this document? Thus, most research in forensic linguistics has focused on speaker identification and author identification. Both speaker and author identification are classification problems; in each, the linguist is seeking a reliable procedure for classifying some speech or some writing to a sample of known speech or known writing.

The histories of speaker identification and author identification show many curious parallels between the voiceprint for speaker identification and forensic stylistics for author identification. Hollien (2002) and Rose (2002) both discuss many theoretical and empirical shortcomings of the voiceprint, a.k.a. aural-visual method, aural-spectrographic identification method. Although academic phoneticians and acoustic engineers rejected the voiceprint, the technique was unfortunately endorsed and used by a prestigious crime laboratory and associated with at least one university. Voiceprint examiners made unbelievable and inflated claims about how many cases they have been involved in, grandiose claims that each human voice is unique and implied that voices were never confusable by voiceprint examiners. The tenacity with which the voiceprint technique lingers, even in the face of empirical evidence repudiating its accuracy and court rulings against its use as testimony is instructive. Author identification has followed much the same path as speaker identification, with forensic stylistics, which is also called by its proponents: text analysis, discourse analysis, sociolinguistics, or psycholinguistics, as the intellectual equivalent of the voiceprint. The world-renowned linguist David Crystal rejected forensic stylistics as linguistics in a review published in *Language*, the prestigious journal of the Linguistic Society of America. Other linguists have also objected to forensic stylistics being represented as linguistics. Some sections of the Federal Bureau of Investigation have adopted and endorsed forensic stylistics, while other sections have recognized the severe limitations of this method and prevented its use as trial evidence. In independent research projects, Chaski (2001, 2007), St. Vincent and Hamilton (2002), and Koppel and Schler (2003) have provided empirical evidence showing that the forensic stylistics method has an extremely low accuracy. Forensic stylistics practitioners have claimed to work in unbelievable numbers of cases, claimed that the each person has a unique set of vaguely defined stylemarkers, and have never produced any empirical evidence in support of their method. Even court rulings which have prevented forensic stylistics testimony from being allowed in trial, or stipulated that the forensic stylistics expert is not an expert in author identification, or restricted the expert so that he can not state an actual opinion about authorship has not stopped the experts from using or attempting to use the method in case and in court.

Meanwhile, there is exciting current computational forensic linguistics research validating methods in both speaker identification and author identification, such as Hollien (2002); Rodman, McAllister, Bitzer, Cepeda, and Abbitt, (2002); Reynolds, Andrews, Campbell, Navratil, Peskin, Adami, Jin, Klusacek, Abramson, Mihaescu, Godfrey, Jones, Xiang, (2003); Rose (2002); Chaski (2005, 2007); Gamon 2004; Stamatatos, Fakotakis, and Kokkinakis (2000, 2001); Diri and Amasyali (2003); Baayen, van Halteran, Neijt and Tweedie (2002). These techniques are meeting the challenges for scientific evidence under both *Daubert* and *Frye*, but still meet resistance in some quarters. Actual reports, court cases, and depositions are presented to support this historical analysis.

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**Forensic Linguistics, Natural Language Engineering,** *Daubert*

## C2    Investigation Methodologies Based on Structural Analysis and Material Mechanical Behavior Correlated With Postmortem Verifications to Reconstruct Events and Acquire Evidence in Forensics

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The goal of this presentation is to discuss technical methodologies to support forensic activities of judges and lawyers. The topic of forensic engineering integrated with forensic medicine expertise will be presented as a unique means to reach the truth.

This presentation will impact the forensic community by demonstrating how the proposed integrated approach, besides the judge for a conscious and objective decision, represents a kind of crossroads among different scientific disciplines. They cooperate equally to the solution of the case, as demonstration that integration strengthens science, giving a practical meaning to theoretical principles. When the judge discusses a case, for which scientific and technical advice is required, he needs both an engineer and a coroner. This significantly increases the chance the truth will be brought to his attention.

As known, forensic engineering consists in applying engineering principles and methods to solution of technical problems in judicial area. A forensic engineer investigates on causes and responsibilities of a harmful event. In a homicide made using varied tools and equipment, as an example, he aids clarifying mechanisms of injuries or death. Also, contribution during disasters (earthquakes, floods, collapses of buildings, air crashes, rail disasters, etc.) has to be mentioned. However, engineering knowledge may not be sufficient to reconstruct events and to acquire evidence in after-the-fact investigations on accidents or failures to be discussed as cases in front of court. A multidisciplinary approach is required and its use is proposed on routine basis.

The purpose of the paper is to demonstrate the usefulness of integration between forensic engineering and forensic medicine. An autopsy can provide information that it is not easy to find in other ways. *Postmortem* tests and verifications can be treated using the engineering traditional means, such as material behavior, stress analysis, Finite Element Method (FEM), etc. Also, simulations made with dummies can be validated with the use of real elements of human bodies acquired through an autopsy.

The proposed integrated approach makes it possible to establish certainties and to speculate about probable hypotheses of event dynamics. A number of cases were reported. They include events like knifing, drowning, shooting, explosion, hanging, car dragging, and accidents at workplace while operating machinery or other equipment. An example is the case where a man head was engaged by a press for scraps. Exhumation of the body allowed highlighting lesions of head and cervical region, confirmed by radiological and histological observations. The correlations among cervical rachis mathematical model, simulations of press working operations, and tests with dummy as well as with foreign bodies (similar to human head) made it possible to establish event dynamics and value of damaging forces applied to cranium and mandible. Another example was the discovery of a dead body in the Po

River. A forensic engineer defined the methodology to calculate the movement of the body both on the bed and the surface of the river.

Since each part of the human body can be assimilated to a machine, bibliography is rich of cases solved using mechanical models. Energy required for generating injuries with side-arms or other improper materials, like glass, can be evaluated. After establishing wideness and deepness of the continuum lesion, biomechanics can also explain the laws regulating the tissular regeneration and the three-dimensional organization of fibers and cells engaged in the *restitution ad integrum*. Expansion pressure of bullets, whether on body surface or inside cavities (cranial, thoracic, abdominal) can be calculated. Linear and angular velocity and acceleration given by an abrupt shaking or a violent impact against a rigid obstacle can be measured. In such accidents finite elements method is applied to the cephalic region (a bond case bounding a cavity containing encephalon, blood vessels, and nerves). Structures involved have dissimilar constitution, so they react differently to traumatic attacks. Crash speed and volume of blood drops, found on the crime scene, can be deducted. The related models help arguments about possible moving of a dead body. Mechanisms and characteristics of lesions in motor vehicles drivers can be studied in detail.

**Autopsy, Investigation, Material**

## C3    Failure Analysis From Fracture Patterns

*Robert N. Anderson, PhD, RNA Consulting, Inc, 27820 Saddle Court, Los Altos Hills, CA 94022; and Anastasia D. Micheals, MS\*, Forensic Materials Consulting, 123 North 25th Street, San Jose, CA 95116*

After attending this presentation, attendees will understand the principals of failure analysis of metal, glass, and plastics that are based on patterns on the fracture surfaces. Attendees will become aware of the difference between fatigue failures and overload failures. In addition, attendees will understand how these principles were applied to the failure analysis of a bio-absorbable screw.

This presentation will impact the forensic community by demonstrating the principles and application of fracture surface analysis and applying these to the failure of a bio-absorbable screw.

The ability to view fracture surfaces has been a tool in analyzing the failure modes in metals. The classic fatigue fracture or overload failure patterns that can be observed visually are known to most investigators who have come in contact with metal failures. The same fracture surface analysis can also be applied to non-metals, providing there is information on the direction the fracture is traveling and thus pointing back to the failure origin.

For metals, stress concentrators such as voids or inclusions can be identified visually or microscopically. These defects can cause overload failure. Beach marks or striations on a fracture surface indicate fatigue. This presentation will present photographs of various fracture surfaces and will discuss its interpretation. The presentation will also review the use of fracture patterns to determine the manner of failure in metals and extends the analysis process to glass and plastics. The specific example of the failure analysis of a bio-absorbable plastic will be given.

Poly (L-Lactide) is a bio-absorbable plastic. Screws made from this type of polymeric material are commonly used in ligament reconstruction. Figure 1 shows such a screw. When a ligament becomes detached from a bone, a substitute ligament or graft is secured into a bone tunnel by use of interference fit bio-absorbable screws. As the graft takes hold and bonds to the bone, the screw is supposed to be absorbed. In some cases, however, the stresses on the screw from manufacturing processes, along with the stresses due to the interference fit cause it to fracture and fail before it dissolves. In one such case of a failure of a Poly screw, images of the fracture surface were taken using a scanning electron microscope (SEM) and the fracture patterns interpreted using surface fracture analysis techniques. The fracture surface, shown in

Figure 2, exhibited Wallner lines. Wallner lines are frequently present in brittle ceramic materials; however, depending on the type of bonding and the elasticity of the material, such features are not always present in plastics. In this case, the Wallner lines indicate the direction of transmission of force and allowed for the determination of the root cause of the failure.
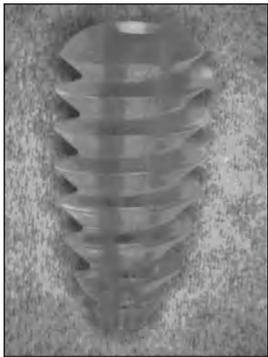


Figure 1: Poly (L-Lactide) bio-absorbable screw
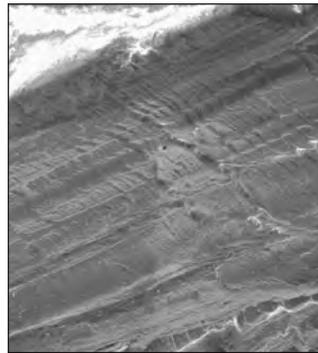


Figure 2: Wallner lines on fracture surface

While the fracture pattern will provide information on the manner of failure of a material, it will not necessarily reveal the root cause of the failure. For example, the failure of an axle may have a fatigue fracture pattern that identifies the point where the fatigue started, but the cause of the fatigue initiation requires more information. Further investigation will be required to determine the root cause, for example a defect or damage at the initiation point causing the fatigue failure to start. If the axle fracture surface shows an overload failure, the fracture surface does not provide information as to where the overload came from, just that the part failed by overload. This information is important in determining whether the failure is either the result of an accident or the cause of an accident.

**Fractures, Fatigue, Bio-Absorbable Screws**

## C4     Analysis of Seat Belt Performance in Rear Impact Crash Testing With Seat Failure

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After attending this presentation, attendees will have a better understanding of the effectiveness of conventional vehicle anchored seat belts when combined with typical weak, collapsing seats during rear impact crash tests.

This presentation will impact the forensic community by showing how older test information, including work published 40 years ago; can still be of benefit in reaching a better understanding of how to protect people in vehicle crashes. The techniques of analyzing older test information, as well as interpretation of that information are presented.

Instrumented rear crash tests from the 1960's through the present were analyzed to determine amplitude and timing of peak vehicle crash pulse and seat belt loads relative to front seat occupant head and chest loads. This was done to determine whether the conventional vehicle-anchored seat belts were effective restraints when combined with typical weak, collapsing seats. Technical literature published in the 1960's and 1970's clearly indicated that conventional seat belts are not effective when vehicle seats collapse rearward. Some of the tests used to make these determinations were unavailable for analysis until recently.

The typical production front seat is far too weak to adequately and safely absorb foreseeable, predictable occupant loads in rear impact collisions. This was recognized by Stapp, Severy, and others in the 1950's and '60's. Typical production seats are so weak that the occupant's head usually does not make contact with the head restraint before the seat collapses. There are also hazards to rear seat occupants as a result of front seat collapse, particularly to children.

Recently, some of Severy's testing from the 1960's period, which was unavailable for public review for many years, was obtained. This has allowed comparison of his data and films with other rear impact research performed for NHTSA and others in subsequent years. Analysis of available test data and films has shown that in conventional collapsing seats, peak occupant head and chest loads precede peak loads on the lap belt in virtually every instance. The only exceptions found to date are where the lap belt is artificially snagged by the pelvis-to-femur joint and/or captured by the molded sitting pelvis of the test dummies. These dummy artifacts are not found in humans. In several tests, belted dummies exceeded Head Injury Criteria acceptable levels, while unbelted dummies did not. It is extremely unusual to have a restrained dummy incur significantly more severe injury than an unrestrained dummy in a conventional linear impact. In addition, the belted and unbelted dummy heads struck the vehicle interior at the exact same millisecond, proving no effective restraint by the belts. In many tests, the peak lap belt loads seen prior to peak head and chest loads, were less than 20 pounds. In an equivalent frontal impact, the peak combined seat belt loads are approaching 2000 pounds or more.

The forensic and biomechanical evidence found in vehicles, especially on seats and seat belts, in field investigations can be correlated to the results of these crash tests.

**Rear Impact, Crash Tests, Seat Belts**

## C5     Seat Belt Forces in Rear Impact Crashes With Seatback Collapse

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After attending this presentation, attendees will understand what happens to an occupant during a seatback collapse and will also understand the magnitude and timing of the belt forces relative to the occupant kinematics and injury causation.

This presentation will impact the forensic community by informing attendees about the forces that develop in seat belts during a rear impact with seatback collapse, the lack of restraining force offered by seat belts in this situation, and hopefully put to rest the misconception that seat belts are effective in seatback collapse accidents.

This presentation examines the seat belt loads for a properly restrained occupant involved in a rear-end collision when the occupant's seatback collapses.

Previous research examined the static effects of occupant girth measurements and slack introduced into the seat belt. The results showed that three-point restraints were not effective in providing restraint when seatback collapse occurs, and that the ease with which the occupant escaped the belt increased with larger abdomen and/or chest circumference. This study examines the dynamic response of the three-point restraint and the occupant during rear-end accidents with seatback collapse.

According to Newton's Laws of Motion, an occupant in a vehicle involved in a crash tends to continue along their pre-impact velocity

vector. That is, in a rear-end impact the occupant must be accelerated by the seat. Relative to the vehicle, the occupant moves rearward into the seatback as the vehicle is accelerated forward beneath them. For a pillar-mounted seat belt, the occupant is moving away from the shoulder harness portion of the seat belt. Under normal conditions, their rearward motion would continue until accelerated to the vehicle velocity by friction with the seat bottom and contact with the seatback and head restraint. When the seatback deforms rearward, the occupant's motion is not arrested and he "ramps" up the seatback. Dangerous "ramping" occurs when the structural failure of the seat increases the angle of the seatback with respect to the occupant. Once the seatback angle changes significantly, the occupant can no longer be fully supported and contained by the seat. Head/shoulder impacts with the rear seat or rear interior vehicle structures, as well as ejection, can occur when an occupant has slid out from beneath their lap and shoulder harness system. Often in these cases, belt usage comes into question.

This study also examines data from a series of 9 sled tests run by Sacalzski and Pozzi. The sled tests were simulated rear impacts with ΔVs varying between 10 to 30 mph. The size of the test dummies ranged from a 5% female to a 50% male weighted to 240 pounds. In these tests the driver's seat was a "weak" seat with a pillar mounted belt while the passenger seat was a much stronger all-belts-to-seat (ABTS) seat. These tests dynamically demonstrate the ineffectiveness of three-point restraints when seatbacks collapse and the resulting injurious occupant kinematics. Belt loads measured during the tests were between 400 and 2100 Newtons. Data plots from these tests clearly demonstrate that the peak seat belt loads occurred 25 to 75 ms after the peak head and neck loads. Thus, the peak belt loads are occurring during the rebound phase of the occupant kinematics, and do not prevent serious head/neck injury resulting from body contact with interior vehicle structures behind the occupant. Data from a frontal NCAP crash test is also presented as a basis for comparison.

Two case studies are presented. In the first case, the driver was ejected when his seat back collapsed and his vehicle subsequently rolled. Seat belt usage was in question. Load marks on the seat belt and photographs from first responders demonstrated that the occupant was initially restrained and slipped out from under his seat belt. In the second case, the belted occupant also slipped under his belt when his seat back collapsed. An analysis with a surrogate occupant in an exemplar vehicle was used in conjunction with the MADYMO computer program to model the occupant kinematics and belt loads experienced during this rear impact where an occupant slipped out from under their restraint. The motion of the occupant in this simulation matched the forensic evidence and statements. The MADYMO results are also consistent with the dynamic test results showing that peak belt loads occur after the peak head and neck loads.

**Seat Belt Loads, Rear-End Impact, Seatback Collapse**

## C6 Determining Vehicle Speed in a Fatal Pedestrian Impact: A Case of Tampered Evidence

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After attending this presentation, attendees will learn a method for determining vehicle speed and orientation for a vehicle-to-pedestrian impact.

This presentation will impact the forensic community by providing a description of a reconstruction method for determining vehicle speed and configuration in some pedestrian impacts.

This presentation identifies and demonstrates a method for determining vehicle speed and orientation in a fatal pedestrian impact. A mathematical dynamic modeling (MADYMO) simulation was utilized to reconstruct the vehicle-to-pedestrian interaction and determine the approximate speed of the vehicle at the moment of impact. The damage observed on the vehicle, as well as the injuries sustained by the pedestrian, allowed for a verified method in determining contact locations of the pedestrian on the vehicle and, thus, vehicle speed for this accident.

This accident occurred when the front driver's side of an SUV made contact with the right side of the pedestrian's head and body. This was a hit and run accident that occurred as a result of driver intoxication as well as driver distraction. According to statements recorded by the investigating police officers, the struck pedestrian was found in a semi-conscious state in a supine position on the side of the roadway. The pedestrian sustained multiple bruises, contusions, and lacerations to the right side of his head, thorax, and pelvic regions. Additionally, treatment at an emergency medical center revealed multiple pelvic and lower spine fractures. As a result of the severity of his internal injuries, the pedestrian later succumbed to excessive internal bleeding and was pronounced dead on the following morning. The pedestrian's point of rest was documented, but because it was not initially determined to be a hit and run accident, firefighters at the scene hosed down the blood on the roadway. The driver of the SUV was not apprehended until a week following the accident. During this period of time, the driver had reportedly instructed a third party to put additional dents on the hood of the SUV, and some repairs had already been conducted by a body shop at the time it was located and impounded. However, an inspection of the vehicle allowed for a distinction to be made between preexisting damage, the intentionally produced damage, and the damage that was related to the subject accident.

The MADYMO simulation was conducted with various critical factors incorporated to provide the most accurate results. The SUV and the pedestrian were modeled independently within the program. Multiple measurements of the front and top of the SUV were recorded and utilized within the MADYMO model of the SUV. A TNO human pedestrian model with a similar height and weight as the subject pedestrian was utilized for the simulation. Because the front and top of the SUV were the areas making contact with the pedestrian, particular care was taken to model the contour, location, and height of these structures.

Once the vehicle and the pedestrian were independently modeled within the simulation program, an iterative approach was taken to determine the position of the pedestrian relative to the roadway. The configuration of the pedestrian relative to the vehicle and the speed of the vehicle were adjusted until the contact locations on the model SUV by the model pedestrian matched the locations of the actual contacts on the subject SUV. A key component in determining the vehicle speed was a dent observed on the roof panel of the subject SUV. Simulations using lower vehicle speeds did not cause the pedestrian to ramp up and contact the roof structure. Thus, the vehicle speed was increased until contact was made in this corresponding location. Because the blunt trauma locations of the actual pedestrian and vehicle damage related to the subject accident were well-documented, it was possible to accurately reconstruct the speed of the vehicle at the point of impact.

In conclusion, when the vehicle-to-pedestrian contact locations are thoroughly documented, this method presented can be utilized to reconstruct the accident, determine the configuration, and the speed of the striking vehicle.

**Pedestrian, Vehicle Speed, MADYMO**

## C7 Seat Failure Evidence in Rear Collisions

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After attending this presentation, attendees will have an understanding of forensic evidence that typically occurs when seats fail during rear impact. This will cover not only various failure modes of seats, but the evidence created by vehicle occupant contact with failed seats and various structures within vehicles.

This presentation will impact the forensic community by showing how to determine if a seat failure has occurred, as well as how a vehicle occupant moved out of their designated upright seating position as a result of that seat failure.

This study involves analysis of evidence created during rear impact laboratory testing of vehicles as well as forensic evidence found on seats and vehicles during field investigations. There have been static and dynamic tests conducted on vehicles, including seats and restraint systems for over 40 years. This includes testing per the minimum requirements of FMVSS 207 and 301-75, as well as research into vehicle crashworthiness and occupant protection effectiveness of various seat and belt combinations. Various types of seat failure, vehicle deformation, and occupant contact witness marks on vehicle structures are demonstrated. Despite the foregoing decades of testing and research proving the need for crashworthy seats and effective restraint systems in rear impact, there still are no government or industry occupant protection standards for rear impact.

The foregoing laboratory studies are compared with field investigations involving rear impacts. Seat failure modes, vehicle intrusion effects, and occupant contact witness marks are analyzed. In several instances there are direct comparisons between evidence found in field investigations and laboratory studies performed to demonstrate the circumstances of the collision. This includes side by side demonstrations of various seat and seat belt designs under identical collision circumstances. These side by side comparisons involve small adult females, average adult males, and heavy adult males. There are also depictions of the effects of these various seat failures on rear seat occupants, including children.

These laboratory and field studies have demonstrated numerous random modes of seat failure in foreseeable rear collisions. One common type of production seat has demonstrated at least seven failure modes. These include failures of seat tracks to floor pan attachments, separation of seat track longitudinal members, disengagement of seat track latches, separation of seat cushion frames from seat tracks, tearing of seat cushion frames, shearing of recliner-to-frame mounting bolts, fracturing of recliner frames, shearing and disengagement of recliner gears, bending of seat cushion frames and seatback frames, tearing of seatback frames, disengagement of adjustment pawls on electrically powered seats, failure of head restraints to remain attached, and pull-out of pin-and-socket hinges.

There is also depiction of fracture and dislocation of plastic rear seats, latch failures of rear seatbacks, and intrusion failures of rear seat structures by cargo. Evidence seen on alternative design seats, including belt integrated seats, is shown.

Seat belt witness marks found during laboratory crash tests and sled tests is demonstrated in conjunction with instrumentation data showing peak belt loads. Dummy contact marks and deformation from head and shoulder impacts into rear seats and other vehicle interior structures is depicted. This includes permanent deformation of rear seat structures correlated with dummy loads.

Comparison of head restraint interaction with occupants in collapsing and non-collapsing seats is shown. Typical collapsing seats show no interaction with occupant head or neck structures prior to seat collapse. This demonstrates that occupants in collapsing seats will typically only interact with head restraints from the mid-thoracic level downward, as they are ramping up the seatback and being ejected rearward.

Drag marks on seatbacks from occupant loading is shown, including correlation with vehicle Principal Direction of Force and evident occupant contact areas. Typical occupant head and shoulder impact marks, skin transfers, hair and fiber deposits, and other related evidence are shown.

Dynamic evaluation of conventional seat belts combined with collapsing seats shows lap belts typically slipping to lower thigh or knee level. Biomechanical studies have shown predictable levels of seat belt slack created by seat back collapse, even under static conditions. Under dynamic loading, these effects are exacerbated. As a result, it is common to find no load marks on seat belts in rear impacts due to the negligible loads imparted by an occupant that is falling away from the belt.

**Rear Impact, Seat Failure, Occupant Witness Marks**

## C8 Multiple Crashworthiness Defects Affecting Vehicle Occupant Survivability in Rear Impact: Failures of Vehicle Fuel System, Seating, and Occupant Compartment Designs

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After attending this presentation, attendees will gain a better understanding of how design and safety decisions made during vehicle development will affect real-world collision outcomes.

This presentation will impact the forensic community by demonstrating how accident reconstruction, vehicle design and crashworthiness analysis, and biomechanics are combined to determine not only how fatal injuries occurred, but why they occurred to restrained occupants in a foreseeable collision.

In most collisions between large and small vehicles, the occupants of the smaller vehicle are normally at far greater risk of injury. In an unusual reversal of this, during rear impacts various safety defects can render the larger vehicle and its occupants more vulnerable than those in the smaller vehicle. This is a case study involving accident reconstruction, biomechanical analysis, and vehicle design/crashworthiness analysis to prove how and why this occurred.

A large utility vehicle containing seven restrained occupants incurred a low to moderate change in velocity after being struck in the right rear by a compact passenger car. The utility vehicle yawed and encountered a half roll as it flipped onto its roof. There was inconclusive evidence of significant roof crush because of subsequent fire damage and heat softening of the roof structures which caused the inverted vehicle to collapse toward the pavement.

The vehicle eventually was consumed by fire as a result of predictable failures of the fuel system. The vehicle had a unique aft-of-axle fuel tank located in the rear crush zone, despite the fact that virtually identical light trucks from the same manufacturer were produced with forward of axle fuel tanks. The justification for this much more hazardous location was to allow six gallons greater fuel capacity. There were significant fuel leaks caused by a failure of the filler neck-to-fuel tank junction, a distortion of the tank near the sender unit, and a hole in the tank adjacent to the greatest tank deformation and folding. Any one of these failures would have created sufficient leakage to support a significant fire. Taken in the aggregate the breaches of the fuel system

created an extremely severe risk of fire. The foregoing was exacerbated by separation of the floor pan in the rear of the vehicle that allowed the leaking fuel to enter the occupant compartment. This same floor pan distortion pushed the rear seat toward the roof of the vehicle, creating significant loss of occupant survival space. The floor pan distortions were as a result of efforts to allow the fuel tank to move up and over the rear axle. The vehicle was never tested with a rear seat in place nor were any test dummies ever placed in any rear seating positions during any dynamic testing.

The driver of the passenger car and the three center rear passengers of the utility vehicle escaped without significant injury, despite the ensuing fire. The three center rear passengers were supported by the legs of the third row occupants, which prevented further collapse of the center rear seatback. However, this created entrapment of the third row seat occupants. The SUV driver and three occupants of the third row seat had no detectable crash injuries, and were conscious and talking after the crash, but died due to the fire. There were predictable failures of seats and restraint systems which affected occupant entrapment and egress from the burning vehicle. Static testing of the seats in this vehicle showed that they were the weakest among all seats tested by NHTSA at that time.

There were also predictable failures of the rear occupant compartment that reduced occupant survival space due to intrusion, and which also greatly increased the penetration of leaking fuel and fire into the vehicle interior. These combined failures were most pronounced at the third row seat area. This case illustrates the myriad of hazards of third row seats in SUV's the vast majority of which are smaller than the vehicle involved in this case. All else being equal, the occupants of other smaller SUV's and vans with third row seats placed near the rear of the vehicle are at extreme risk. Not only are there typically no dynamic tests performed with instrumented dummies in the middle and third row seats, there is no dynamic testing or evaluation of occupant protection in rear impacts or rollovers. Collapse of front and/or middle seat occupants into rear seat occupants is an additional hazard that is not addressed in typical vehicle developmental testing.

This presentation will demonstrate the forensic science involved in determining the foregoing predictable failures in a foreseeable collision involving a very common type of vehicle, and the safety implications for millions of similar vehicles under similar impact conditions. This study especially highlights the emerging safety issues of third row seating in utility vehicles. The design and testing process (and lack thereof) used to develop the vehicle clearly demonstrated the high likelihood of all these failures in foreseeable collisions, prior to initial vehicle production. Static and dynamic testing of the vehicle also demonstrated the predictability of the ensuing multiple fatalities in what should have been a readily survivable collision.

**Rear Impact, Fire, Seat Failure**

## C9    Motorcycle and Rider Kinematics in Low Speed Rear-End Collisions

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After attending this presentation, attendees will understand the appropriate parameters for car to motorcycle rear-end accidents, which can be applied in reconstruction and biomechanical analysis of such accidents.

This presentation will impact the forensic community by offering insight to the motorcycle and rider kinematics in an increasingly more common accident type that has not been previously addressed in the literature.

The results of a series of automobile to motorcycle rear-end impacts using an instrumented motorcycle with an instrumented live human rider in low speed impacts is presented. The unique kinematic patterns associated with rear-end impacts to motorcycles are demonstrated and their usefulness in support of an analysis of a particular motorcycle rear-end accident is shown by example through a case study. In addition, the post-impact drag of the motorcycle with the fender folded in above the rear wheel demonstrated in instrumented tests is also presented.

In response to higher fuel costs there are increasing numbers of commuters choosing to ride motorcycles and scooters.[1] As a natural consequence, a corresponding increase in collisions involving motorcycles can be expected. Thus, the occurrence of a relatively uncommon collision type, a car rear-ending a motorcycle, can be expected to become more common. A review of the literature did not reveal any previous demonstrations of motorcycle or rider kinematics in low speed rear-end collisions.



Figure 1: A BMW R1100RT on the left that was rear-ended by a Ford Crown Victoria shown on the right.

The BMW rider described seeing the approaching Ford, picking up his feet, and then his BMW was pushed forward perhaps 5 or 6 feet while the fender was tucked under against the wheel. His hands did not come off the handle bars.

The Ford's driver described rolling slowly forward while looking away and then hard braking upon the realization that the BMW was stopped. The Ford bumped the BMW at no more than 2 mph and the rider was jostled and did not lose grip of the handlebars. Afterward the vehicles were separated by the distance of an arm's length.

A BMW R1200RT motorcycle was used for both the drag tests and the rear-end impacts. A rider wearing a helmet, riding jacket and boots was on the BMW during both test series. A Mazda 929 was used as the striking vehicle in the rear-end impacts.

The rollout distance indicated by the witnesses was correlated to the BMW's post-impact speed by estimating the BMW's drag. The drag force was measured with a load cell attached to the BMW's forks. The drag was measured to be 12 pounds with the BMW free rolling and balanced by the rider, and the drag was measured to be 36 pounds while the fender/mud flap was tucked against and rubbing on wheel.

Accounting for the combined weight of the motorcycle and rider, the corresponding deceleration of the BMW while free rolling and with the fender/mud flap rubbing against the rear tire were 0.015 g's and 0.05 g's, respectively.

Using the latter deceleration, the account of approximately 3 feet of post-impact rollout of the BMW corresponds to a post-impact speed of about 2 mph, while the account of about 5 or 6 feet of post-impact movement corresponds to a post-impact speed of the BMW of 3 mph. Therefore, the witness accounts of the BMW's movement after impact corresponds to a post-impact speed generally within the approximate 2 to 3 mph range.

Next, a series of automobile to motorcycle impacts were conducted. The additional weight of the data acquisition equipment roughly offset the weight of the BMW's rear fender/mud flap and saddle bags, which were removed to prevent damage to those structures.

The Mazda's speed was measured and recorded using a Racelogic VBOXIII which collects speed data accurate within 0.06 mph at 100 Hz using the Doppler shift of GPS satellite signals. The acceleration of the BMW, rider's head, upper thorax and lower torso were measured by triaxial accelerometers that were affixed to a level portion of the BMW's tank, the front of the rider's helmet, as well as the rider's upper thorax at the approximate forward projection of T1 and the lower torso at the

approximate location of L5-S1. The acceleration data was recorded with a Diversified TDAS rack with two signal input modules strapped to the luggage rack. The acceleration data was collected at 10,000 Hz and SAE J211 sign conventions were observed.

Using a CAS weight scale, which has a 10,000 per wheel capacity in 5 pound increments, the test weight of the BMW was measured to be 585 pounds. Thus, the total weight of the BMW with the rider was about 800 pounds. Similarly, the Mazda 929's test weight was measured to be 3680 pounds.

The time of contact was signaled to the data collection equipment via a tape switch mounted across the front bumper of the Mazda and across the rear wheel of the BMW. The contact closure also activated a flash affixed to the Mazda's hood to provide a visual signal of time zero.

The BMW rider was about 5 feet, 7½ inches tall and weighed approximately 200 pounds. The rider wore boots, a riding jacket, and a helmet. The rider was aware of the testing protocol and was aware of the impending impact since the approaching car was visible in the BMW's rear view mirrors.

A series of 7 impacts were conducted with contact speeds ranging from 1.1 to 4.6 mph. In no test did the rider loose his grip on the handlebars.

| Run | Impact speed (mph) | Rollout (in) |
|---|---|---|
| 1 | 1.1 | 21 |
| 2 | 1.9 | 2 |
| 3 | 2.4 | 5 |
| 4 | 4.2 | 71 |
| 5 | DL | 19 |
| 6 | 3.8 | 12 |
| 7 | 4.6 | 66 |

Table 1: The test series

The BMW bounced away from the Mazda in the 1.1 mph impact resulting in a rollout of 21 inches. The two subsequent impacts resulted in significantly lesser rollouts due to the interaction between the Mazda front bumper and wheel, which tended to retard the motion of both the motorcycle and the car. This significant restriction to forward movement also occurred at higher speeds in runs 5 and 6. In other runs, the rollout was limited to 5 or 6 feet by rider brake application.

Starting at run 3 through the last run, the Mazda's front wheel notably rode up the BMW's rear wheel. This upward motion continued until the Mazda's hood directly contacted the BMW's exposed rear frame below the luggage rack producing hood denting. In run 7, both the Mazda's front wheels lost contact with the ground.

Throughout the demonstrations, rolling of the BMW's wheel while in contact with the Mazda's bumper also produced abrasions through the paint and into the plastic of the bumper cover. With contacts limited to the BMW's rear wheel and fender frame, the motorcycle was undamaged throughout the test series.



Figure 2: Wheel penetration into bumper and hood to frame contact

As seen in figure 3 below, the motorcycle's crash pulse was longer than a typical bumper-to-bumper impact. Throughout the series, the duration of the BMW's acceleration was measured to be within the 130 to 270 msec range, and its peak acceleration ranged from 0.5 to 3.75 g's.
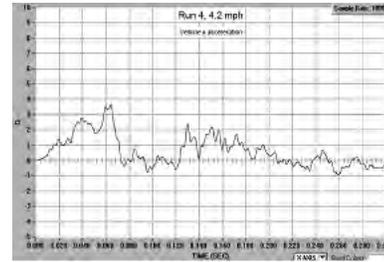


Figure 3: Motorcycle accelerations in Run 4 (4.2 mph)

The longer crash pulse was due to both the softer pole-type contact between the motorcycle wheel and the Mazda bumper, as well as the tendency for the Mazda bumper to ride up onto the BMW's wheel, both of which lengthened the distance over which the BMW was accelerated forward.

In addition to the relatively long duration, high speed video of the final demonstration run reveals that the motorcycle pitched nose-up, further lengthening the distance the rider was accelerated forward.
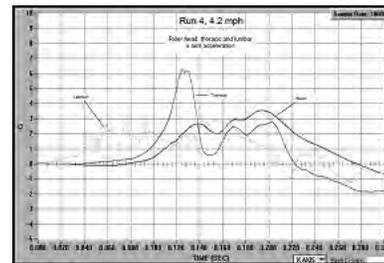


Figure 4: Rider accelerations in Run 4 (4.2 mph)

The rider's buttocks sliding rearward on the seat spread the acceleration of the lower torso over an extended distance, as evident in the long flat lumbar acceleration shown in Figure 4. Also evident is the upper thorax accelerating forward primarily through the rider's arms. The initial peak in the thoracic acceleration corresponds with the rider's arms becoming straight.

Both the sliding of the rider on the seat and the straightening of the arms serve to further lengthen the distance over which the rider as a whole is accelerated forward, thereby reducing the rider's acceleration. The rider's peak head acceleration ranged from 1.2 to 3.6 g's and the average acceleration of the BMW and rider were less than 2 g's throughout the test series. The type of secondary or rebound motion characteristic of car-to-car rear-end impacts was not observed.

Reference:
[1] "Cycle Sales Powered by Pricey Gas," William M. Welch, *USA TODAY*, 6/5/2006.

**Motorcycle, Rear-End, Kinematics**

## C10 Misinterpretation of Data From Linear Accelerometers in Dynamic Vehicle Testing

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After attending this presentation, attendees will understand what an accelerometer is and how it is used in testing; will understand the difference between linear and angular acceleration; and will know the difference between a vehicle's fixed coordinate system and a world coordinate system.

This presentation will impact the forensic community by informing attendees about the proper use and limitations of linear accelerometers and the proper technique for analyzing the data from tests.

This presentation examines data from linear accelerometers collected during dynamic vehicle testing. Currently, there is an ongoing problem with opinions being made from linear accelerometer data where the effects of angular accelerations and gravity are neglected.

Linear accelerometers are used in dynamic automotive testing, including crash testing and dynamic handling tests. SAE J211 is the standard that dictates the proper use of accelerometers and data recording in these tests. SAE J211 assigns a vehicular origin that is assigned near the vehicle's center of mass. This is defined as the vehicle's fixed coordinated system, where all measurements are made relative to the vehicle's center of gravity. For a world coordinate system, measurements are made relative to a point fixed to the earth.

Linear accelerometers do not measure angular acceleration. Because linear accelerometers can only measure in one direction, the accelerometers go off axis and produce questionable data if the vehicle experiences any angular acceleration. This is due to the fact that the origin (the vehicle's center of gravity) is rotating with the vehicle and, therefore, any rotation cannot be accounted for relative to the world coordinate system. Thus, linear accelerometers experiencing angular accelerations will record erroneous results. This is especially relevant if the recorded acceleration is integrated for change in velocity. Change in velocity for a body decelerating is the difference between an initial velocity and a final velocity. With a vehicular origin, it is not known where the change in velocity originates or stops.

In order to use linear accelerometers for data acquisition in dynamic testing with angular acceleration, certain parameters must be met. First, an outside reference must be established to coordinate the vehicle and world coordinate systems. Next, to account for angular accelerations, two linear accelerometers must be set at a known distance. The angular acceleration can then be obtained by calculating the difference between the two accelerometers and multiplying times the distance between them.

Accelerometers measure acceleration in terms of gravity (G's). This makes them susceptible to errors introduced when they go off axis and pick up accelerations due to gravity. This is not significant in a dynamic crash test where there are extremely high G loads. However, in dynamic handling tests, where one is dealing with very low G-loads, the effects of gravity can have a very significant effect. Therefore, a single linear accelerometer cannot be used in dynamic handling tests. To compensate and correct for gravity, rate gyros that measure roll and pitch must be used for accurate data.

An examination of two cases studies was performed. In these cases, data recorded from linear accelerometers mounted at the CG of a vehicle was misinterpreted and flawed opinions were formed. In the first case, use of the wrong coordinate system combined with a disregard for angular accelerations resulted in a 26% error in calculated velocity for a 62 mph vehicle into barrier test. In the second case, the effects of gravity were disregarded when there was pitch or roll of the accelerometer. The second case involved vehicle handling tests where recorded values from accelerometers are typically less than 1 G. The additional acceleration gained or lost by acceleration due to gravity resulted in readings one and a half to two times what they should have been. A static test was conducted to demonstrate this error. A stationary accelerometer was tilted off axis so that gravity was acting on the measured axis. In this demonstration, gravity made it appear that the stationary accelerometer was actually moving.

**Testing, Linear Accelerometers, Case Study**

## C11 Reliability of Time-Motion Surveillance Images as Evidence in Accident Causation: Case Study Involving Images of a Vehicle/Pedestrian Collision

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After attending this presentation, attendees will see that surveillance images, recorded at an accident scene, can in some cases bias an observer of the images as to what actually happened during the course of the event.

This presentation will impact the forensic community by demonstrating how surveillance images can bias the interpretation of the facts of a recorded event. It will show that jurors can be mislead into an incorrect conclusion about an event when they simply view the sequence of images as if it were a movie or video.

Surveillance cameras are very common in today's world. Many businesses and government buildings have a multitude of security cameras that continuously record scenes where people are going about their daily business. As a result, it is quite common for accidents to be recorded by a security camera and for the resulting images to become critical forensic evidence for reconstruction and for a demonstration to the jury as to how the accident occurred.

The subject case study involves a surveillance camera located in a parking lot adjacent to a cross-walk on a public road where a pedestrian was hit by an accelerating van. The camera system recorded a sequence of digital images of the event. The subject of this investigation is to address the degree to which the surveillance image sequence is reliable as forensic trial evidence on the issues of the motion of both the pedestrian and the van during the course of the event. It was found that when simply played back as a video in real-time, the image sequence demonstrated both perspective and temporal distortion when compared to what a hypothetical witness at the scene would have observed.

**Perspective Distortion**: It is well known by professional photographers, photogrammetrists, and imaging scientists that the effect of using a telephoto lens at a relatively long distance produces in the resultant photograph, when viewed normally, a perspective distortion known as "foreshortening", where an object dimension or the distance between objects appears unrealistically small. Conversely, when using a wide angle lens the opposite occurs.

For the subject case, a site inspection revealed that the surveillance camera's distant location and relatively long focal length lens produced a foreshortening effect that biases the viewer of the images toward perceiving the distance that the pedestrian travels before being struck by the van is shorter and therefore his speed slower than they would have perceived if they were eye witnessing the same event.

**Temporal Distortion**: The subject surveillance camera system recorded quarter frames (320 X 240 pixels) at a framing rate of about 4 images per second. Playback at 4 frames per second is significantly below the critical flicker frequency of the human eye, giving the impression of an extremely "choppy" motion that is commonly referred to as a stroboscopic effect or a flicker effect. For reference, the framing rate of a motion picture film at the theater is 24 images per second and

typical home video is recorded at 30 frames per second. Playback of a sequence of images at 4 images per second distorts the perception of motion for the viewer into a choppy sequence of discrete images with visual information between images being non-existent. This is especially problematic, in the subject case, for the reliable perception and judgment of how fast the pedestrian was running or how rapidly the van was accelerating. At this slow framing rate, it is impossible for the viewer of the images to tell if the pedestrian's legs are making a running or walking motion.

**Case Details**: When the surveillance images are viewed as if a movie, and without the benefit of having visited the site of the event, it gives the impression that the pedestrian crossed the intersection at a "fast walk" and in plain view of the van driver who then accelerated into him. The impression is that the van driver had plenty of time to see the pedestrian crossing and should have avoided hitting him by waiting for him to cross before accelerating.

Photogrammetric analysis of the images in conjunction with visual observations and measurements at the site reveal a different story. The camera was found to be located 15 feet up on a pole that was 471 feet from the point of impact and covered a horizontal field of view of only 18.4 degrees (35mm equivalent of a 215mm telephoto lens). It was found that the pedestrian "ran" approximately 50 feet in 3.25 seconds (15.4 ft/s or 10.5 mph) from when he stepped off the curb to when he was struck by the van. Though the viewer of the images sees the pedestrian for a full 4.25 seconds before he is struck, photogrammetric analysis also determined that, for the van driver, the pedestrian was obstructed from view by a vehicle in an adjacent lane until less than 1.25 seconds before the van starts moving and only 2.0 seconds before impact.

The impact of this presentation is to show that surveillance images can bias the observer into concluding facts that are not true. Though the old adage that "the camera never lies" may be true, it is equally true that the observer of the images can easily misinterpret what the camera has recorded.

**Surveillance Camera, Image Analysis, Accident Reconstruction**

# C12    Determination of Free-Phase Conditions Using Ethylbenzene/Xylenes Ratios

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After attending this presentation, attendees will learn how to determine petroleum hydrocarbon free-phase conditions and identify source areas using ethylbenzene/xylenes ratios.

This presentation will impact the forensic community by providing a cost-effective method for determining site-specific free-phase screening levels for releases of gasoline and middle distillate petroleum products in soils that is based on soil types and contaminants present rather than the product that was released, is easily applied using typically available VOC data, and applicable to single and mixed overlapping release scenarios where ethylbenzene and xylenes are present. Additionally, mapping the EXR distribution at sites can identify release areas and soil migration pathways.

The presence of petroleum hydrocarbon (gasoline and middle distillates) free-phase conditions in soils is determined using an evaluation of xylenes and total volatile organic compound (VOC) concentrations to ethylbenzene/xylenes ratios (EXRs). Soil data exhibiting biodegradation from Michigan Leaking Underground Storage Tank (LUST) sites are presented that demonstrate EXR values correlating with free-phase conditions fall within a narrow range and thus are identified. Xylenes concentrations are shown to predict total VOC levels and those having EXR values at levels above the narrow

range indicative for free-phase conditions are used to determine site-specific soil saturation concentration (Csat) screening levels.

"The Csat value is the concentration in soil at which the solubility limits of the soil pore water, the vapor phase limits of the soil pore air, and the absorptive limits of the soil particles have been reached. Csat is a theoretical threshold above which a free-phase liquid hazardous substance may exist. Free-Phase Liquid is a substance that is liquid in its natural state, is not dissolved in water and is at a concentration sufficient to allow it to flow and migrate in the environment separate from the influence of water." (Michigan Department of Environmental Quality (MDEQ) Operational Memorandum No. 1, Technical Support Document - Attachment 8, Part 201 Csat Concentrations, Part 213 Tier 1 Csat Concentrations, 2007).

"Bulk liquid hydrocarbons which coat soil particles are much less subject to environmental alteration, including biodegradation, than hydrocarbons at lower concentration uniformly dispersed in soil or dissolved in groundwater. This is because physical alteration processes mainly affect the interface and not the body of the bulk product. Biodegradation inside the body of the free product is extremely slow, because of the limitation of oxygen, water and nutrients (Hoag and Marley, 1986). It is likely that a hydrocarbon fuel in such conservative environments can survive for a very long time (over 20 years in some cases) with only minor changes in chemical composition." (www.dpra.com/index.cfm/m/160). Free-Phase Liquids represent long term sources of contamination because they will continue to supply contaminants to the environment and replace those which are transported away from the source area, are biodegraded, or removed through remediation (Alexander, 1999).

Gasoline, kerosene, diesel, and heating oil have composition ratios of ethylbenzene to xylenes of approximately 0.20±0.05. Upon a release, typically aerobic bacteria rapidly use the available oxygen and drive the release environment to an anaerobic condition. Ethylbenzene and xylenes are $C_2$ benzene compounds that have nearly identical boiling points, vapor pressures, water solubilities, and carbon-water sorption coefficients. Therefore, the major fate and transport mechanisms of evaporation, water-washing, contaminant velocity retardation, and mechanical dispersion affect ethylbenzene and xylenes alike. Anaerobic biodegradation will remove xylenes faster than ethylbenzene (Reinhard, Hopkins, and LeBron, 2005) and the EXRs will increase with time. An EXR of 0.25 or greater is an indication that anaerobic biodegradation is occurring. (Smith and DeWitt, 2006). Free product or free-phase conditions will act as continuing sources having extremely slow biodegradation resulting in continued elevated contaminant levels in soils and EXR values typically less than 0.25.

Ethylbenzene and xylenes soil data that exhibit anaerobic biodegradation decay are used to evaluate free-phase conditions and determine site-specific Csat screening levels. A graph is presented of the distribution of EXR values for over 100 cumulative soil samples that were observed from multiple LUST sites in Michigan, where xylenes concentrations were detected at levels above the MDEQ Tier 1 Csat screening level (150,000 μg/Kg). The Tier 1 Csat EXR distribution exhibits a general range of 0.07 to 0.24, extending down to 0.03 and up to 0.33, with a mean and median of 0.18. This observed range is consistent with the range predicted for petroleum products (0.20±0.05) and, therefore, is indicative of free-phase conditions.

Field examples are presented of 1) xylenes and total VOC soil concentrations versus EXR and 2) total VOCs versus xylenes for two LUST sites in Romulus (predominantly clay soils and gasoline, diesel, and kerosene potential sources) and Detroit (predominantly sand soils and gasoline and diesel potential sources), Michigan. Graphs are displayed for the combined clay/sand, clay, and sand soil types observed at each site. These field examples show that the higher contaminant levels, which include the MDEQ Tier 1 Csat values, fall within a narrow EXR range between 0.10 and 0.24. The steep onset and decline character of the distribution defines this narrow EXR range that is consistent with

that predicted for petroleum products (0.20±0.05) and thus, is correlated with and identifies free-phase conditions. EXR values greater than approximately 0.25 are consistently associated with lower contaminant levels indicating free-phase conditions are not present at these locations and that anaerobic biodegradation is progressing.

The EXR data and map distribution for the Southfield, Michigan LUST site (gasoline, diesel, and fuel oil potential sources) is presented, which indicate a release area in the northeast portion of the site. Results for the predominantly clay soils show the typical character of the higher contaminant, free-phase EXR values falling within the narrow range generally between 0.15 and 0.26. The lower free-phase EXR values (0.15 to 0.26) are generally clustered in a former dispenser area in the northeast portion of the site indicating a historic dispenser release. The EXR data shows increasingly higher values to the southwest and south, where measurable biodegradation progresses at the lower contaminant levels. The extension of the lower EXR values to the south of the former dispensers and coincident with the former water and gas utilities in this area suggests a migration pathway was facilitated along these utility corridors.

Graphs of total VOCs versus xylenes show a direct linear correlation indicating that xylenes concentrations are a good predictor of total VOC concentrations. EXR values greater than typically 0.25 are associated with progressive biodegradation and lower contaminant levels, indicating free-phase conditions are not present at these locations.

The xylenes concentrations for these data are used to statistically determine a site-specific Csat screening level. The site-specific xylenes Csat screening level is then used to calculate a site-specific total VOC Csat screening level, using the linear equation fit to the total VOCs versus xylene data. The site-specific total VOC Csat screening level is applicable for screening data to indicate free-phase conditions are not present where ethylbenzene and/or xylenes are below the laboratory detection limits or at lower contaminant levels within the elevated, narrow free-phase indicative EXR range below approximately 0.25.

In summary, the EXR method for determining site-specific Csat screening levels for releases of gasoline and middle distillate petroleum products in soils is based on the soil types and contaminants present rather than the product that was released, is easily applied using typically available VOC data, and applicable to single and mixed overlapping release scenarios where ethylbenzene and xylenes are present. Additionally, mapping the EXR distribution at sites can identify release areas and soil migration pathways.

**Petroleum Hydrocarbons, Free Phase, Ethylbenzene and Xylenes**

# C13 Asbestos and Environmental Crimes

*Peggy J. Forney, BS\*, United States Environmental Protection Agency, Office of Enforcement and Compliance Assurance, Office of Criminal Enforcement, Forensics, and Training, National Enforcement Investigations Center, Building 25, Box 25227, Denver Federal Center, Denver, CO 80225*

After attending this presentation, attendees will see how environmental crimes can be proven to have happened even after most of the evidence has been removed.

This presentation will impact the forensic community by showing some of the interesting challenges faced by an environmental forensic chemist in proving beyond a reasonable doubt that violations of environmental laws in the renovation and demolition of asbestos-containing materials (ACM) has occurred.

EPA regulations come into play when specific amounts of ACM are removed from a building or released into the environment. Often when the investigation starts, much of the material has been removed. Calculations from information in photographs or evidence found after the removal may be used to determine if a violation exists.

Several cases will be discussed:

Case #1 involved photos of a pile of debris dumped on the side of the road. Five samples were taken for analysis. Neither the height nor width of the pile was measured, but the analyst was asked to determine how many pounds of asbestos were in the pile. Paperwork found in the pile of debris led back to the company that dumped the asbestos containing material. The company paid a substantial fine and also paid for the cleanup.

Case #2 involved a school that contracted for asbestos pipe wrap removal during the summer vacation. Glove bags were specified in the removal of the wrap from the pipes. Instead, the pipe wrap was ripped off the pipes onto the floor, with the debris later shoveled into the glove bags. The bags were sealed as if used properly, and then disposed. The school was cleaned after abatement and EPA was not called until six months later. The investigator found several small samples of pipe wrap (each smaller than a paperclip), which were analyzed and found to contain asbestos. Vacuum cleaner bags and a vacuum cleaner used a year after the abatement were inspected for asbestos fibers and found to be contaminated, showing that the asbestos debris was spread to multiple classrooms in the school.

Case #3 is where abatement occurred inside a building, but samples taken outside the building showed that the lack of containment contaminated the outside windows and sidewalks. The business was open during the abatement, plus a high school junior/senior prom was held inside the building.

Case #4 involves a casino renovation where drywall coated with asbestos-containing paint was ripped out of the rooms, then illegally dumped into a landfill. The analyst was asked to determine how many pounds of asbestos were deposited into the landfill, using the quantity of asbestos in the paint in a known area on the drywall and photos and measurements of the pile of drywall that was dumped.

These are common types of requests asking the chemists at the U.S. EPA to use not only chemistry, but logic and math to help demonstrate violations of an environmental regulation act have occurred. Not only is polarized light microscopy used to determine the amount of asbestos in samples, but other documentary evidence, such as photos, are used to piece together information for case development.

**Environmental Forensics, Environmental Crimes, Asbestos**

# C14 Microscopy of Soot Particles

*James R. Millette, PhD\*, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Suite 400, Duluth, GA 30096*

After attending this presentation, attendees will understand the basics of soot particle formation including the three most common forms: char, cenisphere, and aciniform. They will be shown what these forms look like and how microscopy might be used tell them apart.

This presentation will impact the forensic community by increasing the general knowledge of how microscopic level soot particles may be similar or different when generated by different combustion sources. These differences may be useful when investigating potential criminal arsons and in environmental forensic investigations of potential industrial contamination and the sources of darkening agents.

Soot is generated during the incomplete combustion of organic materials. Individual soot particles show different characteristics depending on the nature of the fuel and the parameters of combustion. The particle characteristics of the two most common forms of soot, char soot and aciniform soot, can be used to distinguish between different sources. Commercial varieties of soot like carbon black, lampblack, and coke have distinctive characteristics that often allow them to be

distinguished from the non-commercial forms of soot. The ASTM Standard Practice D6602-03b provides a basis for investigations involving soot particles. Using this Practice, polarized light microscopy (PLM) can be used to differentiate between soot particles and other dark particles that may be present in a sample. PLM and scanning electron microscopy equipped with energy dispersive x-ray spectroscopy (SEM-EDS) can be used to differentiate certain forms of coal and coke from other particles of char. Because aciniform soot particles are composed of aggregates of primary particles in the nanometer range, transmission electron microscopy (TEM-EDS) is used to confirm its presence and provide diagnostic characterization. Aciniform soot particles can be classified according to their morphology (shape and appearance of the primary particles and aggregates), their elemental composition, and primary particle size distribution. TEM is also useful in looking at turbostratic layering, an attribute of the internal structure of some primary aciniform particles.

A number of reference samples of soot or similar black carbon materials were obtained and characterized. Standard reference petroleum coke obtained from the National Institute for Standards and Technology (NIST) – (SRM 2718) was found to have similar characteristics to standard reference bituminous coal (NIST-SMR 2693) when analyzed by PLM. They could be differentiated based on elemental composition differences as determined by SEM-EDS. Additional work was also done on reference samples of coal (anthracite, bituminous, lignite) and peat obtained from the American Coal Foundation, Washington, DC. Reference ASTM carbon blacks (grades N134, N220, N326, N330, and N660) were compared with NIST standard reference Diesel Particulate Matter (SRM 2975) using the TEM-EDS procedures described in the ASTM Standard D6602-03b. Commercial carbon black and diesel soot are both composed of aciniform carbon particles. They both show turbostratic layering in their primary particles. Carbon blacks have average primary particle diameters (related to the specific grade) with fairly tight standard deviations. Diesel soot particles can have average primary particle diameters that are similar to carbon black but have larger standard deviations. This presentation will also include illustrations of microscopic characterizations of soot particles that were used in a number of investigations including those concerning California wildfires, industrial combustion sources, candles in residences, and a site of a suspected arson.

**Soot, Microscopy, Aciniform**

## C15 Variation of Refractive Index and Elemental Composition Within a Mineral Wool Product

*Richard S. Brown, MS\*, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Suite 400, Duluth, GA 30096*

After attending this presentation, attendees will understand the terminology of glass fibers and the variation of elemental composition between fibers in a single product sample.

This presentation will impact the forensic community by showing how single mineral wool fibers can not be easily classified as a single glass fiber product.

Using glass fibers as trace evidence presents quite a challenge to the microscopist. The optical properties and the elemental composition of mineral wool fibers (non-continuous glass fibers made from rock (basalt) and/or slag) can vary considerably within a single insulation product. Relying on the optical and elemental composition of one fiber (or even several fibers) found as trace evidence can lead to an incorrect determination as to the mineral wool product type and its source of manufacture.

After attending this presentation, attendees will gain an understanding of the types of glass fibers that are manufactured as insulation and routinely found in everyday residential dust. The definitions of glass or vitreous fiber products that are manufactured and used as insulation will be explained. Examples will be presented that show the overlap in optical properties and elemental compositions amongst glass fibers that are different products with those manufactured from different starting materials. The differences in the reported elemental composition between slag and rock wools (slag and rock wools are mineral wools manufactured from rock and from slag) over the last forty years will be presented. One explanation for the reported differences is the large overlapping range in the elemental compositions of slag wool, rock wool, and mineral wool glass fiber insulation products. The range of optical properties and elemental composition of mineral wool glass fiber insulation products purchased and measured recently will be presented.

Mineral wool, slag wool, and rock wool fibers are excellent insulating materials that are found in ceiling tiles, paper-backed roll insulations and blown insulation products. Glass fibers do not burn and they have not been linked to carcinogenic activity since the beginning of their manufacture. Slag wool and rock wool fibers have optical and elemental properties that overlap between mineral wool product types making the classification of single fibers found as trace evidence as a particular product type difficult, if not impossible. A case study where the iron content of mineral wool fibers was considered as a marker for the classification of mineral wool fibers originating from the destruction of the World Trade Center towers will be presented as an example of just how difficult the characterization of these fiber types can be.

**Mineral Wool, SEM-EDS, PLM**

## C16 Time for Change? The Science & Technology Behind Firearm Trigger Mechanism Evaluation

*John R. Nixon, MBA\*, Athena Research & Consulting, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will gain an appreciation of the basic science and technology behind firearms trigger mechanism evaluation, current trigger mechanism evaluation techniques, the influence that test techniques and their results have on final conclusions drawn, and the ultimate impact of those conclusions within the criminal and civil justice systems. Attendees will learn that the most commonly applied laboratory trigger examination techniques generate inaccurate and incomplete data, and that they frequently result in misleading conclusions being drawn with regard to firearm safety. Attendees will be introduced to a scientifically valid trigger mechanism evaluation technique that has been adopted by crime laboratories and firearms manufacturers worldwide.

This presentation will impact the forensic community by raising awareness of a scientifically valid trigger testing technique and associated data analysis. The implications for civil and criminal litigation, where accidental or negligent discharge of a firearm is an issue, will be discussed.

**Summary, Hypothesis, and Proposition:** The majority of crime laboratories offer a firearms evaluation service to their clients. This paper outlines the science and technology behind firearms trigger mechanism operation, evaluation, and data analysis; and presents an overview of the most commonly employed evaluation techniques. A scientifically valid trigger mechanism evaluation technique will be explained by way of example, and the results will be compared and contrasted to those of commonly applied evaluation techniques. Case studies will be used to illustrate the key points made. It is recommended that current laboratory trigger test protocols be modified to take account of the new technology.
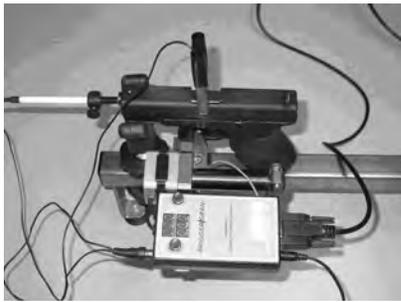
Many jurisdictions prohibit legal possession of firearms by their citizens, however, the criminal elements in those jurisdictions appear intent on ignoring the regulations, and firearms related crime continues to be a problem in many countries. Forensic firearms examination is a routine daily activity in U.S. crime laboratories.

Firearms have been in Western civilizations for over six hundred years, and have evolved significantly over the past two hundred years. Until the last one hundred and fifty years or so, firearms were generally incapable of good accuracy, and were used primarily as short range and/or area weapons (volley musket fire). The advent of rifled barrels, and improved ammunition, resulted in significantly improved accuracy potential. In order to utilize this new accuracy potential it was necessary to develop trigger mechanisms that required less effort to actuate, thereby enabling the shooter to maintain a steady aim.

The effort required to discharge a firearm is a function of both the force applied to, and the distance travelled by, the trigger. Engineers have long been able to design trigger mechanisms and calculate the travel, peak force, and total effort required to discharge a firearm. Engineers have had the capability to scientifically test trigger mechanisms to determine the force-travel profile, and calculate the effort required to actuate the mechanism. However, until relatively recently the techniques involved were labor intensive and required a laboratory. Analysis and interpretation of the test data required appropriate engineering and mathematical education and training.

In the absence of an engineer and a laboratory, firearms users and armorers wanted a simple way to evaluate 'trigger pull' and, historically, the arsenal weight and spring gauge techniques have been used to determine the peak force required to actuate a trigger and thereby discharge a firearm. These peak force techniques, by their nature, generate minimal and inconsistent data. The peak force techniques of trigger evaluation provide an apparently quick and easy method of trigger mechanism evaluation in the field, but the data generated are of very limited value and, in general, are misleading when used to quantify the relative ease of firearm discharge. The peak force test techniques are of value only when assessing whether or not the peak force required to discharge a given model of firearm falls within the normal limits for that firearm. Even this limited application of peak force test data is questionable, because the peak force can fall within specified limits, while other trigger characteristics simultaneously fall outside design specifications.

A purpose built electro-mechanical trigger testing device linked to a computer, and utilizing proprietary software, makes relatively easy work of scientifically valid trigger mechanism testing and evaluation. The technique, equipment, and data analyses are described and explained with the aid of practical examples and case studies. Specifically, the firearm is secured in a test rig, and an electrically driven mechanical arm moves the trigger rearward at constant velocity. The force range is from zero to 20 pounds, with a resolution of 0.007 pounds, and the travel range is from zero to 1.4 inches. Every 0.005 inches the electro-mechanical test apparatus records the force exerted on the trigger face, and these data are downloaded to a computer where proprietary software plots a chart and utilizes integral calculus to determine the total effort required to actuate the trigger mechanism. The software displays key trigger characteristics, such as energy to actuate and trigger travel to actuation. A suitably qualified person can use the chart to visually

identify potential defects, modifications, and/or design deficiencies in the trigger mechanism, and the captured data may be exported and analyzed in greater detail. The widespread application of this test and evaluation technique will have implications for both the criminal and civil justice systems, and these will be discussed.

It is concluded that currently employed peak force methods of firearms trigger evaluation should be phased out and replaced by electro-mechanical trigger evaluation, with the measure of ease of discharge expressed in terms of the effort required to actuate the trigger mechanism, thereby providing a scientifically valid method of comparison between firearms.

**Trigger Pull, Trigger Actuation Energy, Accidental Discharge**

## C17 Forensic Distance Determination by TXRF After Firearm Use

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After attending this presentation, attendees will be introduced to TXRF as a non-destructive technique for gun shot residue analysis with a bench-top system. This paper will demonstrate the suitability of TXRF (Total reflection X-ray fluorescence analysis) for distance determination after firearm use. In contrast to chemical tests, TXRF enables the distance determination for all types of ammunition. In addition, TXRF provides much higher accuracy then the widely used gunshot pattern testing.

TXRF can be applied to different sample types, like solids in form of micro fragments, powders, suspensions, thin films or liquids. The required sample amount is in the low µg or µl range, respectively. In TXRF the samples are prepared as thin film or layer, thus matrix effects are negligible. Quantification is possible by means of the known concentration of an internal standard element.

This paper will impact the forensic science community by introducing TXRF as a versatile and accurate technology, which may replace traditional technologies in most forensic laboratories.

Distance determination after firearm use is an important task during crime scene investigations. This requires trace element analysis of gunshot residue in the area surrounding the bullet hole. The major sources of gunshot residue are the ammunition's primer, which contain lead styphnate, barium nitrate and antimony sulfide compounds. However, some new primers do not contain lead, but can be characterized by other elements (e.g., Cu and Zn). In this presentation the determination of shooting distances for plumbiferrous and unleaded ammunition by TXRF is described.

All measurements were performed using the bench top TXRF spectrometer S2 PICOFOX. Shooting experiments were performed on white scrim. After shooting of textile samples, an area around the bullet hole was cut out. The textile samples were treated with aqua regia prior to the analysis.

A correlation of the element concentrations and the shooting distance for leaded and unleaded ammunition was shown up to a distance of about 45 and 60 inches, respectively.

The results of the presented measurements clearly indicate the suitability of a TXRF spectrometer for distance determination after firearm shooting. In contrast to other analytical methods like atomic spectroscopy, no external calibration is necessary. The simultaneous determination of all detectable elements by TXRF is possible also in case of unknown ammunition or element concentrations. Therefore, TXRF offers the flexibility to handle future changes of primer compositions. Finally, the presentation will give an outlook about further forensic

applications, which will be covered by TXRF in the future. A semi-quantitative element analysis of minute amounts of glass splints or pigment samples followed by correspondence analysis will provide an unambiguous fingerprint of each sample. This will lead to a doubtless identification of the source or manufacturer of such a sample. Forensic scientists will receive the evidentiary value within a minimum of time required for sample preparation, measurement and quantification.

**Shooting Distance, Gun Shot Residue, Elemental Analysis**

## C18 Weight Adjusted Meta-Analysis of Fibrillation Risk From Taser® Conducted Electrical Weapons

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After attending this presentation, attendees will understand the risk of CEW fibrillation for human beings of various body weights.

This presentation will impact the forensic community by demonstrating how a forensic investigator will be able to estimate the risk of CEW-induced fibrillation with an arrest-related death.

**Background**: Some have raised the concern that the rapid pulses from the TASER® X26 Conducted Electrical Weapon (CEW) might induce ventricular fibrillation (VF) from an exposure to the chest. This concern has received some support from occasional reports of the induction of VF in swine with the TASER X26. The TASER M26 has not been suggested as causing VF.[1] The electrical current threshold for VF is approximately proportional to the body weight for both utility power and CEW pulses.[2-4] This has raised the issue of the scalability of these results to heavier humans as the mean weight of excited delirium deaths is 91 kg.[5]

**Methods:** Published peer-reviewed papers studying the application of chest exposures of the TASER X26 to swine were researched — in which the heart was in the current path between the barbs. If individual weights were not given reported ranges to build an appropriate distribution were used. Swine weights were scaled using a moderate correction (human weight = 0.72* swine weight) from the classic Dalziel data[2] even though more recent evidence suggests that swine are even more sensitive to the induction of VF.[6-8] The cases of reported VF induction were then entered along with the exposures not inducing VF into a logistic regression dose-response model. Acute epinephrine effects were scaled using the published 28% VF threshold reduction.[9]

**Results**: Eight papers were found meeting the criteria.[1, 4, 10-15] These studies covered 117 chest exposures in 81 swine weighing between 22-117 kg. There were three inductions of VF in 56 tests with swine of ≤ 37 kg for a probability of .05. There were no VF inductions in swine of > 37 kg. These data were well fit ($r^2$ = .81 by U test) to a logistic regression model (p=.0003 by Wald chi-square test) as shown in the figure. The human weight at which VF induction is likely (p > 0.5) is predicted to be 13.3 kg (confidence limits: 7.1, 21.2 kg). These estimates are conservative as the majority of chest exposures do not include the heart in the current path. The predicted probability of VF in a 91 kg human is $3.4 \times 10^{-12}$ (confidence limits: $4.5 \times 10^{-23}$, $3.2 \times 10^{-7}$).
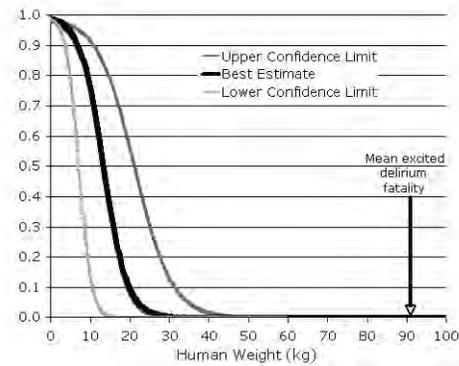


**Conclusions:** Consistent with historical and recent literature, the susceptibility to VF is strongly and negatively correlated with body weight. For human weights < 20 kg VF induction may be possible for successful chest exposures which include the heart between the barbs. The probability of VF with a chest application of a CEW is essentially zero for the weight of the typical excited delirium fatality case. The theoretical possibility of CEW induced VF does not appear to be a plausible explanation for arrest-related deaths.

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**Taser®, Fibrillation, Conducted Electrical Weapon**

## C19    The Effect of PCR Additives and Enhancement Techniques on DNA Recovery From Fired Cartridge Cases and Compromised Samples

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During this presentation, attendees will be made aware of the effectiveness of PCR additives and enhancement techniques on the typing success of artificially-compromised DNA and DNA recovered from fired cartridge cases. These PCR enhancement techniques were implemented during PowerPlex® 16 amplification and STR analysis in attempt to increase the success of developing genetic profiles from touch DNA recovered on fired cartridge cases and other compromised samples.

This presentation will impact the forensic community by determining the value of new methods for developing DNA profiles from fired cartridge cases that can be implemented with currently accepted amplification and typing techniques with minor modifications.

Cartridge cases are often handled by the person responsible for discharging a firearm and recovered at crime scenes. However, the touch DNA that may be present is often thought to be of insufficient quantity or too highly degraded for STR amplification. Prior research at the Virginia Department of Forensic Science (VDFS)[1] has shown that sufficient DNA may be recoverable from handled cartridge cases for STR profiling but its success is limited by the molecular integrity and polymerase inhibition encountered with these samples as a result of the firing process. Partial profiles have repeatedly been generated from fired cartridge cases in the research setting under "realistic" conditions, implicating that optimized PCR amplification and DNA repair techniques may generate profiles with more discriminatory results. Understanding the degree of compromise and the resulting quality of DNA may enable DNA repair or stabilization techniques to be utilized in the preparation and amplification of DNA for STR typing, thereby allowing for increased typing success of such samples.

In this study, DNA was collected from fired cartridge cases handled by a DNA shedder. The quality of DNA recovered from fired cartridge cases was analyzed using Plexor HY™, a quantitative PCR technique.[2] PCR additives including Tween® 20, betaine, dimethyl sulfoxide (DMSO), formamide, and PCRboost™ were evaluated along with the commercial PreCR™ Repair mix, and MinElute® PCR purification kit using artificially-compromised samples including fired cartridge cases. The use of the PreCR™ Repair mix failed to identify a specific form of damage present in fired cartridge case samples. None of the PCR-enhancement additives significantly improved the STR typing results obtained.

Therefore, the results of this study do not support the routine analysis of cartridge case samples with the enhancement techniques evaluated. The studies suggest a large variation in DNA yield and STR typing results with fired cartridge case samples. Further studies are warranted to test non-probative casework samples.

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**PCR Additives, Damaged DNA, Cartridge Cases**

## D1    Homicides Mortality Trends in Puerto Rico — 1999-2007

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After attending this presentation, attendees will learn about the trends of homicides in Puerto Rico during the period of 1999 until 2007. The goal of this study is to increase the awareness of the forensic community, law enforcement, and public health agencies about the fatalities in Puerto Rico due to homicides.

This presentation will impact the forensic community by presenting statistical information about the trends and demographics of homicides in Puerto Rico during the first part of the 21st century including the gender difference, age range, and map distribution across the Island.

The Puerto Rico Institute of Forensic Sciences (PRIFS) serves a population of about 3.9 millions citizens. PRIFS was created by the Puerto Rican Legislature in 1985 and merged the Police Department Criminal Laboratory, the Forensic Medicine Institute of Puerto Rico, and the Bureau of Special Investigation's Technical Service Division of the Department of Justice. PRIFS receives all homicide cases for investigation. For this retrospective analysis, descriptive statistics with mortality rates were used age-adjusted to the Puerto Rican population established by the U.S. Census. The population estimate for each year was used to make accurate comparisons. Mortality rates and trends were stratified by sex and age.

For the period under study (1999-2007), 52,122 cases were analyzed of which 7,154 (14%) were classified as homicides. The number of homicides ranged from 729 (2000) to 838 (2004) with an annual average of 788 cases. The annual mortality rate did not have significant changes. The rate of over 19 homicides per 100,000 is the largest in the U.S. and its territories. The mortality rate for men was statistically higher than women with over 35 homicides per 100,000 for men compared to only 2.4 homicides per 100,000 for women (Figure 1). Eighty-five percent (85%) of homicides in Puerto Rico were committed using firearms with multiple shot wounds (semi-automatic and automatic weapons), followed by 6.6% of stab wounds, 5.3% of trauma, and 1.6% strangulation. In all categories, the percentage of men was

higher (>70%), but for strangulation this percentage was similar, 54.3% for men and 45.7% for women.

The homicides were clustered in the age range of 15 to 44 years of age for both genders (Figure 2). The highest accumulation of cases is in the 20-24 years range with a mortality rate of approximately 600 homicides. Men in this age range had an outstanding cumulative mortality rate of 1,200 homicides. For year 2007, the mortality rate for men in the 20-24 years was 122 per 100,000 range and only 4.2 per 100,000 for women.

The distribution of homicides across the Island shows a pattern towards the northern part and San Juan in particular with a rate of over 40 homicides per 100,000.
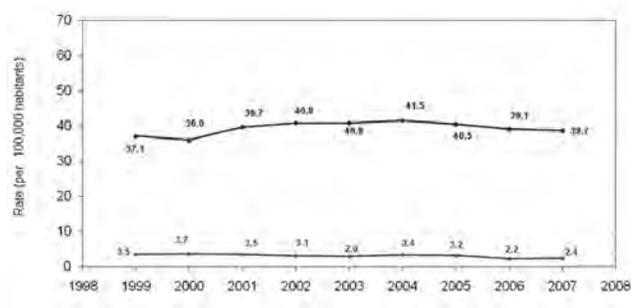


Figure 1. Mortality rate of homicides per gender for the period of 1999-2007 in Puerto Rico.
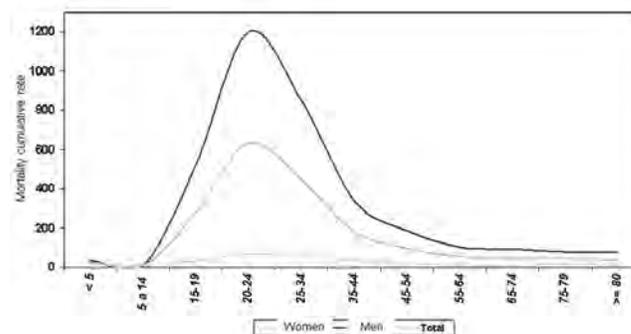


Figure 2. Cumulative homicide mortality rate per gender and age range for the period of 1999-2007 in Puerto Rico.

**Homicides, Puerto Rico, Gender**

## D2    The Changing Role of the Medicolegal Death Investigator Conducting SUID Investigations

*Yvonne Ledesma, BS\*, and Cynthia S. Larson, BS, Miami-Dade Medical Examiner Department, Number One on Bob Hope Road, Miami, FL 33136*

After attending this presentation, attendees will realize how a thorough infant death investigation changed the role of the medicolegal investigator at the Miami Dade Medical Examiner department.

This presentation will impact the forensic community by emphasizing the need for all medicolegal investigators to conduct a thorough investigation to ascertain the entirety of the circumstances surrounding infant deaths and create public awareness of the potential risk factors of unsafe sleep environments.

The Miami-Dade County Medical Examiner Department implemented the Center of Disease Control's (CDC) doll reenactment protocol in February 2007 as a routine component of pediatric death investigations. This change in standard operating procedure for the medicolegal investigator states that all possible Sudden Unexplained Infant Deaths (SUID) must include field-based investigative interviews and doll reenactment with parents and/or caregivers. The caregiver is asked to demonstrate to the investigator the sleep environment in which the infant was last placed and the position in which the infant was found. The requirement of doll reenactments by the CDC has helped the role of the medicolegal investigator to evolve from that of a basic telephone interviewer to an in-field investigator. This change in infant death protocol helps to document unsafe environmental factors such as co-sleeping with companions.

The CDC has found that the decline in Sudden Infant Death Syndrome (SIDS) rates since 1995 has been offset by increasing rates of other types of sudden unexplained infant deaths. A recent review suggests that asphyxia and co-sleeping continue to be significant risk factors in SUID investigations. The new investigative protocol provides photographic and written documentation to inform the forensic pathologist of the first responder's observations, and therefore, provide a more reliable death certification.

Sudden Unexpected Infant Death Investigations (SUIDI) are difficult cases for both families and law enforcement. The doll re-enactment is a tool that the investigators can use to depict possible risk factors. Investigators have accepted their new responsibilities to better assist the pathologist with the determination of cause and manner of death. The majority of cases have shown that caregivers are cooperative with the investigation and perform doll re-enactments to provide the details surrounding the terminal event.

Several area hospitals surveyed verified educational materials regarding possible risk factors are provided to new parents. However, hospital staff expressed their concerns regarding that many caregivers failed to follow the current recommendations.

The Miami-Dade Medicolegal Investigators are professionals who are also taking proactive measures by attending educational seminars and maintaining interactions with law enforcement and the public. The strategic plan for the future is to enhance professional standards by providing further education with the use of visual aids as part of the educational information provided to parents/caregivers regarding risk factors and potential causes of injury and death. This service to the public is an attempt to prevent future incidents and decrease the mortality rates of infants in our community.

**Medicolegal Investigator, Sudden Infant Unexplained Death Investigation, Doll Re-Enactment**

## D3    Fatal Unintentional Injuries Among Young Children — A Study From South India

*Tanuj Kanchan, MD\*, Kasturba Medical College, Department of Forensic Medicine, Light House Hill Road, Mangalore, 575 001, INDIA*

After attending the presentation, the attendees will identify with the pattern and trend of fatal unintentional injuries in young children in Manipal, South India.

This presentation will impact the forensic community by developing an understanding of the burden of fatal unintentional childhood injuries in the region and to develop preventive strategies so that human lives are saved.

Unintentional childhood injuries constitute a significant public health problem which are vastly preventable. The goal of this study is to describe the pattern and trend of accidental deaths in young children in Manipal, South India. This study is a registry based, descriptive research spanning over a period of 14 years from January 1994 to December 2007. All medicolegal autopsy case records were retrospectively reviewed and cases of fatal unintentional injuries in children aged ten years and below were studied. The information obtained from autopsy reports, police investigations, and toxicological analysis was registered in a database and analyzed. Deaths due to suicidal and homicidal manner were excluded.

During the study period, seventy-five cases of fatal accidental childhood injuries were identified. Males accounted for 68% of cases, with the male-female ratio being 2.1:1. Road traffic fatalities accounted for the greatest number of fatalities (52%), followed by those due to thermal injuries (22.7%). Flame was the cause of thermal injuries in 52.9% cases and fatal scalds were observed in 47.1% cases. Traffic fatalities, falls, and drowning were more common in school age children, while toddlers and pre-school age children were relatively at a greater risk from domestic accidents (thermal injuries and poisoning). The highest number of victims in road traffic incidents were pedestrians (64.1%) and head injuries alone were responsible for fatal outcome in 82.1% cases. The results of the study are compared with studies done elsewhere in India and abroad.

Unintentional childhood injuries constitute a significant public health problem which is vastly preventable. The study highlights on the pattern of accidental fatalities among children in Manipal, South India. To reduce the burden of unintentional childhood mortalities, priorities for school age children are traffic injuries, while for toddlers and pre-school children are thermal injuries.

Morbidity and mortality in children can be prevented by understanding common patterns of injury and educating parents and children about injury prevention. Injury risk can be reduced through injury prevention strategies, child education, and family education. Children should be taught to swim and play safely in and around water, and to stay away from fire and hot fluids. Kerosene lamps should be kept away from children. Enforcement of safety regulations by the state and educating parents about potential household poisons so that such agents are kept in secure places and out of the reach of the child, can help reduce unintentional poisonings. Age-appropriate school-based programs should also be developed to address traffic safety and can go a long way in reducing mortality in children. Although improvement in health services is the aim in management of childhood trauma, and better healthcare facilities definitely bring down the mortality rate, the main emphasis must be on prevention if more lives are to be saved.

**Unintentional injuries, Children, South India**

## D4    Sudden Unexpected Infant Death Scene Investigation – National Training Academies Effect on Death Scene Investigation

*Steven C. Clark, PhD\*, Occupational Research and Assessment, 124 Elm Street, Big Rapids, MI 49307*

After attending this presentation, attendees will be able to identify various SUIDI tools and application technologies, locate "experts" within their region for information and training, and identify the role of each new investigative tool in the proper certification of sudden unexplained infant death. Attendees will also understand how to register as users of the national SUIDI registry.

This presentation will impact the forensic community by demonstrating the performance of medicolegal death scene investigators and their ability to communicate scene findings to forensic pathologists

for more accurate cause and manner of death determinations in SUID cases.

SIDS rates have declined by more than 50% since the early 1990s in large part due to the national Back-to-Sleep campaign to increase the proportion of infants being placed on their backs to sleep. Despite this success, SIDS is still the third leading cause of infant mortality in the U.S. and remains an important public health priority. CDC research has found that the decline in SIDS rates since 1999 is offset (or can be explained) by increasing rates of unknown cause of death and other sudden, unexpected deaths in infancy (SUDI). This finding suggests that death scene investigators, and those certifying cause-of-death on the death certificate, have changed the way they have been investigating and reporting these infant deaths in recent years.

To address this issue of change in reporting, there is a need to: (1) standardize the methods used to conduct infant death scene investigations, (2) standardized the data sets collected from infant death scene investigations, (3) create a method of reporting critical data to the forensic pathologist *prior* to autopsy, and (4) establish methods of translating death scene investigation (DSI) findings into consistent cause and manner of death certifications nationally. Standardizing and improving data collection at infant death scene investigations and national reporting of all sudden, unexplained deaths in infancy (SUDI) including SIDS is a national priority recognized by CDC and supported by the highest level of the U.S. government (Congress).

The Centers for Disease Control and Prevention's (CDC) national effort to standardize and improve the quality of infant death scene investigations through the funding for five SUIDI National Train-the-Trainer Academies will be described. Why standard data collection instrument and training materials are important for improving data collection at the scene, national reporting, and evaluation of data, and how each of these essential elements for the prevention of sudden, unexpected infant deaths will be explained. Moreover, the benefits of the new reporting form, electronic reporting system, and training materials for medical examiner/coroner investigators who conduct infant death scene investigations will be explained.

Finally, the presentation will introduce medical examiners and coroners to investigative tools that will enhance their ability to conduct a thorough infant death scene investigation. Namely, the utility of the Sudden, Unexpected Infant Death Investigation Report Form (SUIDIRF), and the new electronic national registry for reporting SUIDI data, and associated training materials will be demonstrated. The training materials will be available in several formats including web-based training, DVD, VHS, CD-ROM, and in-class training manuals.

**Infant Death Investigation, SUID, SUIDI National Academies**

## D5 A Typology of Voluntary Assault and Battery Upon Children Under the Age of 15 and Factors Associated With the More Severe Cases: A Three-Year Retrospective Study in a Lyon University Hospital, France

*Géraldine Maujean, MD*, Laurent Fanton, MD, Hervé Fabrizi, MD, and Daniel Malicier, Institut de Médecine Légale, 12 Avenue Rockefeller, Lyon, 69008, FRANCE*

After attending this presentation, attendees will become aware of a particular type of physical violence perpetrated against children in the last three years in the third largest French town.

This presentation will impact the forensic science community by pointing out the factors associated with the more severe cases of voluntary assault and battery upon children under the age of 15.

**Objectives:** The United Nations Children's Fund research has recently estimated that almost 3,500 children under the age of 15 die from physical abuse and neglect every year in the industrialized world; the greatest risk being among younger children. According to the National Observatory on Social Decentralized Action, almost 19,000 children experienced either physical or psychological violence in 2006 in France. As the only systematically collected data in legal proceedings concerning violence perpetrated in the family, such statisctics were biased and much of the violence against children remained under-recorded. A retrospective study was conducted over three years in Edouard Herriot's Hospital in Lyon, France, to describe voluntary assault and battery upon children under the age of 15 and to investigate the factors associated with the more severe cases.

**Methods:** All children under the age of 15 who were examined in the forensic consultation for voluntary physical or psychological assault and battery between January, 1 2005 and December 31, 2007 were retrospectively included. All victims of sexual assault were excluded. For each case, demographic characteristics, aggression history, medical, and forensic data were collected from medical records according to a standardized data collection form. Victims were classified as severe if the injury prevented normal daily activity for more than eight days. Severe cases were compared to mild cases using Chi2 or non-parametric tests. Multivariate logistic regression was used for risk factors' identification. Statistical analyses were performed with SPSS for Windows, version 12.0.

**Results:** Among the 193 children included (62.2% male, median age of 8.0, range 0.5 to 15), 34 (17.6%) severe cases were reported. The average number of days being prevented from daily activity was 4.75 (range 0 to 45).

Factors independently associated with a severe case after multiple logistic regression were:
- Separated or divorced parents (0R=0.27; IC95% 0.07-1.01; p=0.05)
- Children attending school (OR=0.07; IC95% 0.01-0.44; p<0.01)
- Non-family young offender under the age of 18 (OR=14.2; IC95% 3; 6.7; p<0.01)
- Aggression at home (OR=0.19; IC95% 0.04-0.95; p=0.04)
- Punched or kicked children (OR=0.09; IC95% 0.02-0.46; p<0.01)
- Traumatic wound (OR=18.3; IC95% 2.16-154.7; p<0.01)
- Arm wound (OR=3.9; IC95% 1.07-14.1; p=0.04)

**Conclusion:** Differences between severe and mild case characteristics exist wherever sociological or aggression-related factors are considered. The lower proportion of severe injuries perpetrated at the young victim's home may reflect most physical violence against children in the family, which usually does not cause serious visible physical injury and often requires repeated incidents to allow community-based or legal interventions. Non-family, young offenders under the age of 18 and victims not attending school are two significant factors associated with severe cases. These results first illustrate youth violence which has dramatically increased worldwide in the last two decades without being confined to any one subgroup of the youth population. Finally, this study underlines the importance of schooling and school quality to prevent such kind of violence.

**Physical Violence, Child, Risk Factors**

## D6 Household Furniture Tip-Over Deaths of Young Children

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After attending this presentation, attendees will understand the role of medicolegal death investigations in identifying childhood deaths due to household furniture tip-overs.

This presentation will impact the forensic community by calling attention to household hazards that may be the cause of preventable childhood deaths.

Although many investigators have recognized that unsafe sleeping conditions such as bed sharing (co-sleeping) and/or compressible sleep surfaces play a causal role in many sudden, unexplained infant deaths, there is a dramatic increase in the incidence of accidental deaths when children reach the developmental stage of mobility. Accidental deaths in childhood result from falls, poisoning, drowning, fires/burns, transportation-related deaths, and deaths due to foreign body inhalation. The majority of these deaths occur in the child's residence, and many result from avoidable hazards in the home and/or lapses in supervision of the children by their caregivers, in some instances because of impairment of the caregivers due to exhaustion or substance abuse. Examples include poisonings resulting from a child having access to household products containing hazardous chemicals, or to objects left within the child's reach that could be swallowed, and deaths due to hyperthermia when children are inadvertently left in closed vehicles.

Although the majority of childhood accidental deaths in the home relate to readily recognizable domestic sources of danger such as drowning deaths due to inadequate barrier mechanisms preventing the child from having access to residential swimming pools, other hazards are less well recognized. Childhood deaths due to tip-overs of household furniture or appliances are uncommon. The Consumer Products Safety Commission (CPSC) has warned the public of the potential for injuries and deaths due to pieces of furniture or television sets falling on young children. However, the forensic literature contains little information on childhood deaths resulting from furniture tip-overs.

Nine childhood deaths that resulted from household accidents in which furniture or domestics appliances fell on the child will be presented to elucidate the causes of death in such rare but potentially preventable circumstances. Three of these deaths resulted from bedroom dressers falling onto a child, one from the tip-over of a kitchen stove, one from a lounge chair, and four from television sets. All but one child was less than five years old. The cause of death was attributed to blunt head trauma in three cases and chest and abdominal trauma in one. Four deaths were certified as asphyxia due to chest compression, with the weight of the heavy object impeding the child's breathing. The cause of death in the remaining case was attributed to a combination of asphyxia and blunt head trauma. In all nine cases the death could have been prevented by adequate anchoring of the piece of furniture or by closer supervision of the child.

Childhood deaths due to traumatic asphyxia are uncommon. In these circumstances, the determination of the cause and manner of death must be based predominately on the investigation of the scene and circumstances of death, since the physical findings at autopsy are few and nonspecific. The medicolegal death investigator, in collaboration with the investigating law enforcement agency, plays a key role in elucidating the cause and manner of death in such cases. These findings indicate that a thorough, multidisciplinary approach correlating the scene investigation with autopsy findings is essential in reducing the incidence of deaths due such domestic hazards.

**Death, Child, Furniture**

## D7 Experiential Learning: An Effective Method of Training for Radiographers Involved in Mass Fatality Incidents?

Kim E. Hutchings, MSc*, Inforce Foundation, DCMS, Cranfield Forensic Institute, Cranfield University, Shrivenham, SN6 8LA, UNITED KINGDOM; Mark D. Viner, MSc*, Inforce Foundation, Forensic Science Institute, Cranfield University, Royal Military College of Science, Shrivenham, UNITED KINGDOM; and Roland Wessling, MSc, Inforce Foundation, Cranfield University, Cranfield Forensic Institutde, Shrivenham, SN6 8LA, UNITED KINGDOM

After attending this presentation, attendees will understand the value of experiential learning through the use of simulation exercises in the training of mass fatality responders. Attendees will learn how the creation of scenarios that replicate the elements of mass fatality incidents enable students to develop the necessary skills in a controlled and educational environment.

This presentation will impact the forensic community by highlighting the role and contribution of radiography in mass fatality investigations and demonstrating the value of simulation exercises in training forensic professionals to respond to mass fatality incidents.

**Method:** Typically radiographers (radiologic technologists) work in safe, controlled environments with other professionals who understand radiology, radiation science, and the contribution of imaging to medical investigation. In a mass fatality incident this will not be the case; the situation will be unfamiliar and traumatic. The radiographer will need to act with confidence and speed and be mindful of the situation unfolding around them. They may be the only imaging professional in the team. The use of simulation training in a true multidisciplinary team environment helps the radiographer to gain firsthand experience of a realistic mass fatality situation, to plan and to understand the implications and limitations of their actions, and develop the necessary skills for disaster response within a controlled environment. By creating a simulated mass fatality incident in which all the elements of emergency forensic response are represented, students can experience firsthand the multifaceted challenges presented by such a situation. Students can develop and try out their own strategies for overcoming practical and organizational challenges in a learning environment, supported by a team of experienced tutors. By use of multidisciplinary training exercises, students gain understanding of the challenges faced by other professionals and learn to adopt a team approach to solving practical problems to achieve a common objective.

**Results:** Students learn to:
- Understand the scope of a mass fatality incident
- Adapt to changing circumstances
- Develop the confidence to work as the sole radiation expert in the team
- Contribute effectively to the team
- Establish their own x-ray facility
- Have consideration for the safety of self and others
- Undertake a radiation safety survey and train others in radiation safety
- Participate in the identification process.

**Conclusion:** There is no way to prepare adequately for a mass fatality incident as each and every incident will be different. However such simulation exercises assist students to prepare for, and adapt to, any situation as it unfolds, and to act professionally and confidently as part of a multidisciplinary team.

**Mass Fatality, Forensic Radiography, Simulation Training**

## D8 Comparison of Differential Processing Techniques for Development of Latent Prints on Porous Substrates

*Marissa Olvera, BA, BS\*, 3203 Park Avenue Apartment C, Richmond, VA 23221*

After attending this presentation, attendees will become more informed regarding the performance of Oil Red O as a latent print processing reagent.

This presentation will impact the forensic community by providing additional instruction and information in the maximum development of latent prints.

Friction ridge skin forms on the hands and feet of a fetus in utero and the pattern produced is permanent unless altered as a result of injury or disease. The orientation, location, and relationship of ridge characteristics allow for individualization or exclusion of a fingerprint to its source. An impression of the friction ridge pattern can be transferred during contact with a surface, resulting in a latent print, if invisible to the naked eye.[1] Validation of the use of ORO in the development of latent prints on paper substrates, integration of ORO in sequence with other methods of fingerprint development and carrier solvent examination are important areas of research in latent print analysis. Investigation and optimization of the techniques used for the visualization of latent prints are essential to the successful contribution of latent print analysis in forensic science.

Latent fingerprints are typically composed of varying amounts of salts, amino acids, fats, oils, and waxes. Routine chemical processing techniques in the development of the water-soluble, amino acid component of latent fingerprints include DFO (1,8-diazofluoren-9-one) and ninhydrin (triketo-hydrindene hydrate).[2] The lipid components of latent fingerprints are routinely processed via Physical Developer (PD).[3] The use of Oil Red O (ORO), a lypophilic stain, has been established as being effective in the development of latent prints on paper substrates and as a possible replacement for Physical Developer (PD). Oil Red O (ORO) was evaluated as a latent fingerprint reagent on various paper substrates in comparison to PD and in sequence with DFO, 1, 2-IND and NIN. Performance of 1, 2-IND in contrast with DFO was also assessed. Amino acid based and sebaceous based fingerprints were deposited on nine paper substrates including book paper, sticky notes, brown paper, manila envelope, newspaper, notebook paper, copy paper, cardboard, and check paper. Upon development, each fingerprint was paired with its original partner and visually compared to determine the effect of carrier solvent on sebaceous print development. All comparisons were verified by a qualified latent print examiner at the Oregon State Police Forensic Services Division, Springfield. ORO was found to develop more latent fingerprints of better quality than PD solo and in sequence with other latent fingerprint reagents. 1, 2-IND was observed to have a positive effect on ORO development when used as a replacement for DFO. HFE-7100 was utilized as the carrier solvent for DFO, 1, 2-IND, and NIN as it was demonstrated that fewer fingerprints developed using PD. It is recommended that ORO should be used as a latent fingerprint reagent using the parameters described, in place of PD. HFE-7100 should be used in lieu of PD as the carrier solvents for amino acid based latent print reagents, DFO, 1, 2-IND, and NIN. When utilizing ORO for latent print processing, it is also recommended that 1, 2-IND should be used in place of DFO.

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**Oil Red O, Latent Prints, HFE-7100**

## D9 Oral Forensic Photography Protocol

*Patricia A. Crane, PhD\*, University of Texas Medical Branch Galveston, School of Nursing, 301 University Boulevard, Galveston, TX 77555-1029; and Diana Faugno, MSN\*, 1351 Heritage Court, Escondido, CA 92027*

The goals of this presentation are to demonstrate patient positions to maximize quality of oral images, explain storyboard images of oral photographs and to provide rationale for images and the value of each position.

This presentation will impact the forensic community by demonstrating how penetration of the oral cavity with patient reports of sexual assault requires a unique clinician skill set and unique techniques of photography to provide valuable evidentiary photographs of the oral findings.

Forensic photography requires special skill and techniques when a patient reporting sexual assault discloses oral penetration. Oral images are of great value especially if the patient is not able to provide the history of the event, has no memory of the event, and there were no witnesses. For the sexual assault examiner, photography may also include images of the body and images of the oral and genital area looking for injury, no injury, or evidence of pre-existing medical conditions for documentation. Photography allows sexual assault examiners to add images for enhanced documentation and the opportunity to consult and teach in a peer review setting. Defense experts are also able to view the findings and provide valuable consultation in these criminal or civil cases.

This presentation will focus on providing a guideline or protocol that can guide the sexual assault examiner, or other clinician, through the process of obtaining the highest quality of oral images possible.

The sexual assault examiner typically performs a forensic medical examination, including subjective and objective assessment, and documents the physical findings. Forensic aspects of care require that the examiner provide documentation of injury or no injury for the record. Documentation is provided in written form, diagrams, and photographic images.

A pictorial presentation of the photographic protocol in this presentation will assist sexual assault examiners with providing the highest quality oral examination with patients reporting oral penetration with sexual assault. Such a protocol will assist the examiner in obtaining maximum value from the oral examination and images in order to have complete high quality evidence following a medical forensic examination.

The trend toward digital photography with the magnification software provides extreme detail for all parts of the oral cavity. This is critical in order for the examiner to be able to identify the micro trauma that is typical of sexual assault, including oral penetration. Magnification plays a major role in determining the size of the depth of field range. Patient and clinician positioning for long-range, medium-range, and close-range images will be demonstrated for obtaining the best images of external lips, mouth, soft and hard palates, and other oral anatomical sites. Comparison of images with explanations will be provided to explain to participants the rationale for assuring that images meet the criteria for best quality images for use as evidence in legal proceedings. The expected criteria for high quality images that are expected in the courtroom include: (1) picture is in focus, (2) picture is not too light or too dark, (3) picture is aligned and not twisted, (4) picture truly represents the subject matter, (5) picture is not compressed too

much, and (6) picture has adequate image resolution to visualize what is necessary.

Examples and several images similar in subject matter will be displayed for the participant's visualization of the difference in resolution and quality of picture. The participant will be able to observe and compare the quality of images and apply the information to their own sexual assault practice.

**Oral Photography, Sexual Assault, Forensic Imaging Documentation**

## D10 Molecular Palynology Study in Central East Texas: A New Approach to Linking Crime Scenes

*Jamie L. Jouett, BS*, Sam Houston State University, 13 FM 1696 E, Huntsville, TX 77320*

After attending this presentation, attendees will see how molecular palynology in combination with a Geographical Information Systems (GIS)-based analysis may help to link pollen samples collected at a crime scene to or from a suspect with a particular geographic area and the associated vegetation.

This presentation will impact the forensic community by demonstrating a correlation between STR (Short Tandem Repeat) analysis of pollen/plant DNA and geographical location to potentially link pollen evidence to a crime scene.

Forensic palynology is gradually becoming a more recognized scientific field as the analytical technology has developed to the point that trace evidence, such as pollen collected from a crime scene or suspect, can be characterized efficiently. Pollen evidence has been successfully used in the past to solve criminal cases; however, no initiative has been taken to merge hi-tech analytical techniques and mapping programs such as GIS with DNA analysis. Similar to humans, plants and pollen are comprised of DNA. STR analysis of plant and pollen evidence could provide the missing link, which allows differentiation among pollen evidence, in turn, narrowing the window of searching to either include or exclude geographical areas from the scope of a criminal investigation.

The objective of this study is to demonstrate how pollen collected at a crime scene or from a suspect can be linked to a geographical location by STR analysis and sequencing samples of pollen DNA. A wide range of pollen samples were collected from the northern, southern, eastern, western, and central areas of Huntsville, TX. This presentation illustrates geographical profiling by mapping the Huntsville area and associated vegetation using GIS software. In addition, a new molecular approach involving STR analysis of DNA was performed on each collected pollen sample. These were compared to reference samples to identify a species and subsequently link the pollen sample back to a plant source. The steps in this method involve DNA extraction, amplification by PCR, and detection by STR analysis. In doing so, specific pollen from a native plant species is characterized by establishing a unique DNA profile.

The ultimate goal of this study is to increase the availability of pollen data and stress the significance of pollen collection at crime scenes. Thus, by broadening the knowledge of forensic scientists, further opportunities, initiatives, and new methodologies and advancements in the forensic palynology field will be realized. Most importantly, the study and ideas presented here provide a basis for STR analysis of pollen DNA and will subsequently benefit forensic scientists in criminal investigations involving pollen/plant evidence. Moreover, the application and software described above will set the benchmark for generating a database combining pollen DNA profiles with geographical locations.

**Palynology, Short Tandem Repeat (STR), Geographical Information Systems (GIS)**

## D11 Evaluation of a Prototype Field Deployable Device for Rapid Acoustic Analysis of Liquids and Contraband in Opaque Containers

*Cheryl L. Brophy, BS, Federal Bureau of Investigation, Counterterrorism Forensic Science Research Unit (CFSRU), 2001 Investigation Parkway, Quantico, VA 22135; Morgan A. Turano, MALS, 71 Spy Pond Lane, Arlington, MA 02474; Aaron A. Diaz, BS, Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, WA 99352; and Brian A. Eckenrode, PhD*, Federal Bureau of Investigation, CFSRU, Building 12, Quantico, VA 22135*

After attending this presentation, attendees will learn of a prototype device that is being tested for its ability to be deployed in the field for acoustic, non-invasive analysis of containers of varying sizes and the ability to detect contraband.

This presentation will impact the forensic community by optimizing acoustic field devices used to acquire safe, rapid, and accurate data from containers which will prevent dangerous or illicit substances from penetrating transportation networks and border entry sites.

In the ongoing efforts of the Federal Bureau of Investigation to characterize and block terrorist threats to multi-modal and air transport systems of the United States, research has been accomplished to generate a field deployable device which acoustically interrogates containers of various sizes and material composition, and responds with quick, real-time identification of potential threat liquids and/or contraband.

The beta version of this portable device has been evaluated for performance via examination of ultrasonic time-of-flight measurements, attenuation responses, comparative studies of liquid and dry transducer couplants, and the device's ability to discern the presence of foreign bodies hidden in containers. Upon determination of the operational stability of this prototype device, a goal is to be one step closer to bringing a non-invasive, user friendly instrument to the field that will provide quick assessment of threat liquids and/or contraband, without the concerns associated with destructive or discharging methods.

The protocol by which the device was evaluated included use of containers fabricated from five materials found in the "stream-of-commerce". Three sizes of each of the five container wall types were employed, to vary diameter and thus the acoustic time-of-flight responses within the same liquid type. Four commercially available liquids were used to determine if the instrument would identify the contents and respond with velocities and attenuations that could be compared to literature values and/or to independently measured baseline values.

A Phase I study was completed and results indicated that improvement was achieved by changing the transducer couplant material from wet to dry mode, such that all RSD's for velocity responses were less than 1%. Additionally, the transducer dry couplant material was found to be more robust with daily, consistent use. It was discovered that improvements in the area of distance acquisition from caliper readings were necessary for this prototype, and such improvements have already been implemented in a currently designed bench top analog at PNNL. Phase II studies will include expanding the database for precursor and threat liquids. Obstruction analysis was also performed using various materials, of varying shapes and sizes, placed within each container type and liquid type, as previously described.

It is the intent of this evaluation to assist those interested in deploying an acoustic field device to reach the goal of acquiring safe, rapid, and accurate data which will help prevent dangerous or illicit substances from penetrating transportation networks and border sites. Insights will also provide direction for a Phase II evaluation/modification of the device.

**Ultrasonic, Acoustic, Liquid**

## D12 VICTIMS: A National Database Solution for Unidentified Human Remains in the United States

*Philip N. Williams, BS\*, Federal Bureau of Investigation Laboratory, CFSRU, Building 12, Quantico, VA 22135; Lisa Bailey, BA, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, SPU/Room 1115, Quantico, VA 22135; and Melissa A. Torpey, MS, Federal Bureau of Investigation Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory, CFSRU, Federal Bureau of Investigation Academy, Building 12, Quantico, VA 22135*

After attending this presentation, attendees will become aware of the existence and usefulness of the FBI VICTIMS software system. In addition this presentation should promote the use of the VICTIMS system as a forensic tool for identifying unidentified human remains.

This presentation will impact the forensic community and the public by demonstrating how VICTIMS will create an environment capable of assisting a variety of forensic professionals in melding their data for the analytical comparison between missing persons and unidentified human remains records, as well as an environment where friends and families of missing persons can easily search all of the unidentified records in the United States for their loved one in the pursuit of making an identification.

After attending this presentation, attendees will learn more about how they and their agencies can contribute to the VICTIMS (Victim Information, Catalog, Tracking, and IMage System) database, how to enter and maintain their unidentified records within the VICTIM System, and the benefits of utilizing VICTIMS. Currently in its final phase of development, the VICTIMS software is designed to remedy a number of problems with existing approaches to solving cases of unidentified human remains. The goal of VICTIMS is to provide a national database of unidentified human remains that will organize and coordinate the efforts of the forensic identification community and the public.

Records of unidentified human remains cases have been available on the FBI website since its inception. However, these records were neither comprehensive nor searchable. As a result, in 1998, the FBI Laboratory embarked on a focused effort to solve the increasing number of cases involving unidentified human remains. While the FBI has made improvements in a number of forensic fields (DNA, facial reconstruction, etc), these improvements have largely been conducted in isolation within the specific fields in which they apply. In order to bridge the gap between the various forensic disciplines that assist with the identification of unidentified human remains, a centralized, comprehensive, and role-based software system is currently in development at the FBI. The role-based atmosphere will allow the ability to isolate and protect all data elements for all users based on who they are and what they can contribute to the system and the identification process. VICTIMS is designed to be a comprehensive and internet-accessible environment for the collection, storage, indexing, searching, and retrieval of all forms of data that might assist in the identification of unidentified human remains. The collected data types include (but are not limited to) photographs, facial reconstructions, anthropological reports, medical examiners reports, radiographs, text, case data, and NCIC data that are pertinent to assisting in identification. Many of these data forms have never been available in a searchable electronic format or viewable by the public.

**VICTIMS, Unidentified Human Remains, National Data**

## D13 What Does a Forensic Entomologist Really Do?

*Ralph E. Williams, PhD\*, Purdue University, Department of Entomology, 901 West State Street, West Lafayette, IN 47907*

After attending this presentation, attendees will be shown how forensic entomology is often used in forensic investigations, especially in death investigations. There have been some misunderstandings as to what a qualified forensic entomologist can actually provide. This presentation will spell out how forensic entomology contributes in the forensic investigation as to estimating the PMI and other aspects.

This presentation will impact the forensic community by providing the forensic community a clear understanding of the role and value that forensic entomology can provide in forensic investigations. A qualified forensic entomologist can play an important part of the investigation in helping to determine the PMI, habitat location, linking a suspect to the crime scene, and other aspects.

In defining what constitutes being a forensic entomologist it should first be understood the definition of the term forensic. The Webster Dictionary definition states "belonging to, used in, or suitable to courts of judicature or to public discussion and debate." In the broad sense then, a forensic entomologist deals with legal aspects in which insects may play a role. Death investigation, food contamination, wound invasion, civil suits/litigation involving insect nuisance complaints/infestation, among others, are situations in which a forensic entomologist may get involved.

In death investigations, the forensic entomologist can provide expertise in assessing the postmortem interval, possible site(s) of trauma, geographic location of death, toxicology of the decedent, and identity of the victim via DNA. These can be accomplished by a trained forensic entomologist because there is published information available as to the known succession of insect fauna on cadavers, known life histories of the most common forensically important species, and known geographical and climatic zones and conditions for various species.

With insects involved in food contamination, wound invasion, and civil/litigation cases, a forensic entomologist dealing in these areas has access to similar published information as to the insects that may be involved.

Discussion will focus on these areas that forensic entomologists may deal with and why they are asked by crime scene investigators, coroners, medical examiners, and attorneys to be involved and/or provide expert opinion on various cases.

**Forensic Entomology, Forensic Entomologist, Postmortem Interval**

## D14 Historical Human Remains Identification: Skeletal Analysis, Facial Reconstruction, and DNA Analysis of Alleged James-Younger Gang Member

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After attending this presentation, the attendees will understand: (1) the use of skeletal analysis, forensic reconstruction and DNA testing for establishing personal identification in an historical case, and (2) the use of comparing published articles and newspaper accounts as documentation in the absence of antemortem medical records for establishing personal identification.

This presentation will impact the forensic community by demonstrating how case review, investigation of information, and evaluation of different identification methods can be used to establish personal identification of historical human skeletal remains.

An expanded investigation of a historic case was conducted in an attempt to identify human skeletal remains. The alleged skeletal remains of Charley Pitts, a James-Younger Gang member who participated in the 1876 bank raid in Northfield, Minnesota were donated to the Northfield Historical Society in 1981. In 1982, the remains were examined by a medical examiner from Hennepin County, Minnesota to determine if the skeletal remains could have been Pitts. In 2007, the Historical Society submitted the remains to Minnesota State University, Mankato for further investigative and forensic analysis in search of additional information about their identity.

An examination of historical records, articles, documents, and interviews was included in the investigation. A forensic analysis was also conducted which included an anthropological examination, a forensic facial reconstruction from a computer tomography (CT) scan of the skull, and DNA analysis by three labs; the Netherlands Forensic Institute Laboratory and two private laboratories in the United States.

Pitts' fatal injury was described as a gunshot wound between his $2^{nd}$ and $3^{rd}$ ribs approximately one inch to the left of the sternum. He suffered a buck shot injury in the right arm approximately five inches from the shoulder and another in the back, approximately five inches from the hip. He was described as 5'9 ¾" in height with straight black hair, a stubby mustache and short black beard. His body was transported to St. Paul, Minnesota and Dr. Frank Murphy, Surgeon General, embalmed the body. After its sojourn in the State Capitol for two days of public viewing, no verifiable evidence of the disposition of Pitts' remains has been located. Purportedly, medical students had the remains made into a medical study specimen. By the mid-1950s, the Stagecoach Museum in Shakopee, Minnesota displayed a skeleton alleged to be Pitts and in 1981 donated the skeleton to the Northfield Historical Society. However, some question the authenticity of the alleged Pitts skeleton since the Stagecoach Museum owner demonstrated creative showmanship skills in operating the reproduction western style town.

The general condition of the purported Pitts skeleton is the same as it was when the Northfield Historical Society received it as a donation. It is professionally assembled with wire and pins and some bones are connected with brass fittings. Approximately 95% of the skeleton is complete.

In the anthropological examination, gender determination was based on sexually dimorphic characteristics, overall size, and robusticity of elements. Based on the assessment of skeletal features, the skeleton exhibited male characteristics. The age, approximately 35-40, was estimated using the pubic symphysis, ectocranial suture closure, sternal rib end morphology, and auricular surface. Even though some of these methods are more accurate for estimating age, each was examined to arrive at an estimated age.

Ancestry was determined by examination of the skull and facial features. The skeleton's characteristics suggest primarily Caucasian ancestry with some Asian admixture. Stature was determined using the Trotter-Gleser technique. Measurement with the lowest error rate, that of the femur and tibia combined, was used to calculate the individual's height, which would have been approximately 5' 7". The skeleton was also examined for physical evidence of trauma and pathological conditions affecting the bones.

To produce a facial restoration, computer tomography (CT) files of the skull were converted to stereolithographic (STL) files. From the STL files, a copy of the skull was cast. Erasers indicating skin depths were attached on strategic anatomical landmarks and photographed on the Frankfort Horizontal Plane. Sketches were drawn and the skull was covered in modeling clay and sculpted for the finished forensic facial reconstruction.

For the DNA analysis, bone samples and three teeth were removed from the skeleton. A cross-sectional sample from the mid shaft left femur, weighing approximately 30g and one molar were submitted to the Netherlands Forensic Institute for DNA analysis. Also, two longitudinal sections of femur weighing approximately 30g each and a single molar were sent to each of the private laboratories.

Descriptive data for Pitts was obtained from published articles, newspaper accounts, family interviews and postmortem photographs. In addition, the forensic facial reconstruction from the CT scan of the skull was compared to known photographs of Pitts to determine resemblance. In the identification process, the DNA extracted from the bone and teeth was compared to DNA from Pitts' great grandnephew. The analysis of the skeletal remains, facial reconstruction, and DNA were evaluated to establish whether the skeleton belongs to Charley Pitts or whether it should be eliminated.

**Skeletal Analysis, Facial Reconstruction, DNA Analysis**

## D15    Estimation of Postmortem Interval by Morphological and DNA Changes of Blood

*Amal A. Mashali, MD\*, Alexandria University, Faculty of Medicine, 20 Syria Street, Rousdy, Alexandria,  EGYPT; and Maha A. Ghanem, MD, 51 Victor Amaneul, Semoha, Alexandria, 21615, EGYPT*

After attending this presentation, attendees will see the effect of time on the cellular morphological changes and DNA degradation which occurs in blood after death at variable time intervals.

This presentation will impact the forensic community by demonstrating how the estimation of the time of death is one of the most important problems in forensic medicine and law. Most experienced forensic pathologists agree that the ordinary postmortem changes are easily influenced by external factors. A variety of procedures are used for the purpose of postmortem interval estimation including the analysis of postmortem blood for various biochemical substances.

In the present study, postmortem blood samples were examined to demonstrate the effect of time on the cellular morphological changes and DNA degradation which occur in blood after death at variable time intervals. The study included 30 blood samples from autopsy and dead hospital cases, where light microscopic examination was used to study the morphological cellular changes of blood. Postmortem DNA changes were also studied using gel electrophoresis as well as flowcytometric analysis.

At six hours postmortem the neutrophils, oesinophils, and monocytes shared in showing pyknosis of the nucleus as a starting sign of white blood cell degenerative changes. At 18 hours postmortem, only the neutrophils and oesinophils started showing nuclear fragmentation, whereas, the monocytes didn't show this change until 24 hours after death. Disintegration of the neutrophils, oesinophils, and monocytes began to appear at 48 hours postmortem.

Gel electrophoresis was used in the present work to assay the integrity of DNA within the studied blood samples. Up to 18 hours after death, gel electrophoresis revealed that the majority of cellular DNA was intact. Starting from 24 hours postmortem until 72 hours, DNA fragmentation progressed where it began to smear in tracks indicating the presence of degraded, low molecular weight DNA as well as high molecular weight DNA. Upon reaching day three most of the DNA had been degraded to low molecular weight fragments.

Histograms obtained by flowcytometry revealed that autopsy and dead hospital samples showed similar patterns of DNA degradation after death with no significant difference observed. The values of degraded DNA increased gradually over different postmortem intervals, whereas the values of normal and double DNA content decreased gradually. A significant positive correlation was observed between time since death and the pattern of DNA degradation based upon the flowcytometric analysis of the studied samples. The resulting equations for estimation of postmortem interval from DNA content of cells measured by

flowcytometry revealed an acceptable degree of accuracy in accomplishing this goal

**Postmortem Interval, Blood Morphology, DNA**

## D16 Under the Radar — Into the Forensic Pathologist's Domain: Recognition of the Deceased Victim of Human Trafficking

*Sharon R. Crowley, MN\*, 122 Emeline Avenue, Santa Cruz, CA 95060*

After attending this presentation, attendees will gain an increased understanding of the dynamics of human trafficking. Some of the key indicators and physical manifestations, which may delineate a victim of human trafficking will be discussed. Attendees will also learn about an innovative trafficking first-responder program, part of a long-standing anti-trafficking program in San Francisco, CA.

This presentation will impact the forensic community by increasing awareness of the scope of the problem of human trafficking, improving the understanding of the etiology and manifestations of trafficking cases with a fatal outcome, and encouraging and promoting collaborative relationships with other professionals involved in eradication of trafficking.

Modern-day slavery exists in virtually every country of the world, including the United States. Everyday, individuals are held in domestic servitude and exploited for commercial sex. Current estimates by the annual State Department's *Trafficking in Persons Report*, estimate that 800,000 people are trafficked across international borders each year. Eighty percent are female; half are children. According to Ambassador Mark Lagon (2008), these numbers do not include the millions who are trafficked within national borders for the purposes of labor and sexual exploitation. The demand for cheap labor and commercial sex has created an industry that is tied with the illegal arms trade as the world's second largest criminal enterprise. Trafficking is the fastest growing (Health and Human Services).

Because of the high incidence of forced prostitution, it is timely to take initial steps at recognition of the victim who dies, either directly, or indirectly, as a result of the consequences of human trafficking. Efforts are being made nationally to heighten awareness among health care providers, of both the scope of the problem and recognition of health indicators.

Because so many trafficking victims are enslaved and exploited sexually, it may be difficult to discern the bigger picture. Numerous myths abound about prostitution. In a statement by the Bureau of Affairs, U.S. State Department, where prostitution is legalized, there is a greater demand for human trafficking. The vast majority of women in prostitution neither chooses nor wants to be there. Most are desperate to leave. Females and males who engage in prostitution are often targets of opportunity for criminals. Whether they engage in street prostitution or work in massage parlors, which effectively function as brothels, victims of trafficking are highly vulnerable. Closed brothels may operate out of private residences; these further isolate the working victim. Isolation, cultural separation, language barriers, and often an inherent fear of the police, may cause victims to miss opportunities to escape their dire situations. If they die, their deaths may go unnoticed as trafficking-related. The very measures that keep these individuals enslaved protect the traffickers. In order to begin to study how to better recognize the fatal victim of human trafficking, it may be helpful to explore lessons learned from programs that effectively interact with living trafficking victims. While a great deal is below the radar, much has been learned.

In San Francisco, California, one stellar program stands out for its unparalleled approach to intervention and prevention efforts. In 1992, *Standing Against Global Exploitation (SAGE)* was founded by Norma

Hotaling. SAGE's mission is to bring an end to commercial sexual exploitation and restore the lives of women and girls who are survivors of, or at risk of sexual exploitation and violence. The average age of the trafficking victim that reports to SAGE is the mid-twenties to mid-thirties; however, some SAGE clients may come for services that have been trafficked at some time in the past. The youth component of SAGE is targeted for 12-17-year-old clients, with an average age of 15-years-old.

California is a major entry point for human trafficking. Forty-three percent of the incidences in California occur in the San Francisco Bay Area. The majority of international trafficking victims seen at SAGE come from Asia and South East Asia, especially rural areas in the Philippines, Korea, China, Japan, Thailand, and Vietnam. The second largest source is Latin America, especially Peru and Mexico. Domestic trafficking constitutes approximately half of the clientele. For domestic trafficking clients seen at SAGE, preliminary proportions for race are as follows: African-American (55%), White (29%), Hispanic (12%), Asian (2%), and Other (2%).

In November 2007, SAGE launched the *Rescue and Restore, Reclaim Your Rights* campaign, an intense anti-trafficking effort in San Francisco, supported by Health & Human Services, Office of Refugee Resettlement. In January 2008, SAGE initiated the first of its kind, *Trafficking First Responder Team*, in partnership with the United Way of the Bay Area. Through a series of public service announcements and outreach materials, potential trafficking victims may be linked directly, 24 hours a day, with trained first responders, via information and referral specialists that have been trained by SAGE staff.

Nationally, Health and Human Services, Administration for Children & Families (ACF) actively promotes its *Rescue and Restore Campaign*. Legislative efforts include the 2000 *Trafficking Victims Protection Act*, which is designed for both U.S. citizens and non-citizens alike.

Additional, brief, succinct assessment, and documentation may prove extremely valuable in discerning and tracking fatalities due to human trafficking. Based on field research in nine countries on prostitution, the State Department concluded that few activities are as brutal or damaging as prostitution: 60-75% were raped; 70-95% were assaulted physically. A startling 68% met the criteria set for post-traumatic stress disorder. This was in the same range as combat veterans and victims of state-organized torture (Farley, Journal of Trauma, 2003).

In addition to malnutrition, physical abuse, and sexual assault, other public health implications include diseases such as tuberculosis, syphilis, HIV/AIDS, pelvic inflammatory disease (PID), and other sexually transmitted diseases. In discussing a five-country prostitution study, Raymond et al (2002) concluded that the extent of physical injuries and illnesses of women in the sex industry was overlooked. Prostitutes suffer higher rates of hepatitis B, greater risk of cervical cancer, fertility complications, and psychological trauma. Physical manifestations of these health problems may appear at autopsy and serve to raise the index of suspicion.

While the deceased victim cannot present a thorough history of victimization, certain findings, such as lifestyle information, where known, may prove useful. This may be especially true when combined with "missing" data, such as lack of identification, passport, or visa. The presence of blunt force trauma, perhaps in areas of the body normally covered by clothing, and indicators of sexual assault on genital examination may warrant further investigation. In living trafficking victims, some additional red flags may be a suspicious interpreter, or "friend", and disparity in the living conditions between victims and traffickers. If a massage parlor or business address is investigated, hidden luggage or other evidence may show that the victim/employee lived there. Food, dishes, and toiletries may be present.

Further study is needed to determine if there are additional markers or variables to help differentiate this special, uniquely vulnerable population of victims. In order to eradicate this shameful practice,

successful collaboration between those who serve the living and the deceased can only serve to be of immeasurable value to both, but ultimately to the victims.

**Human Trafficking, Fatal Sexual Violence, Prostitution**

## D17 Death and Disability Due to Delayed Airbag Deployment

*LeRoy Riddick, MD\*, University of South Alabama Medical Center, Department of Pathology, 2451 Fillingim Street, Mobile, AL 36617; and B.G. Brogdon, MD\*, University of South Alabama Medical Center, Department of Radiology, 2451 Fillingim Street, Mastin 301, Mobile, AL 36617*

After attending this presentation, attendees will learn of mechanisms of injury from late airbag deployment, which was the proximate cause of disability and death in this accident. The value of a multidisciplinary approach is emphasized in elucidating the split-second concatenation of several design, structural, and operational failures required to produce this copiously illustrated calamity.

This presentation will impact the forensic community by demonstrating the value of assembly of multidisciplinary groups of forensic scientists in the analysis of complex events.

To avoid a vehicle entering the highway from a side road, a 1997 Infiniti Q45 four-door Sedan swerved into the oncoming lane. This caused an offset head-on collision with a 1996 2-door Chrysler Sebring. All occupants in both vehicles were belted and the frontal airbags deployed. Of the six occupants of the Infiniti, only the driver was injured with a broken leg and big toe. The driver of the Sebring was dead at the scene and his daughter, age 9, in the front passenger seat sustained a depressed skull fracture and brain damage.

Why this enormous disparity in severity of injury to the two sedans? The working hypothesis was delayed airbag deployment in the Sebring.

An array of experts — automotive engineers, a blood splatter specialist, a forensic pathologist, a forensic radiologist, and other medical specialists — were assembled to analyze and reconstruct the accident and its sequelae.

The driver's compartment, markedly reduced by intrusion of the engine compartment and left front wheel well, entrapped him between the seat back, the airbag, and the steering wheel. The driver was not autopsied after the accident, but on exhumation, had compression injuries of the chest with multiple postero-lateral left rib fractures, lacerations of the lung and diaphragm, and hemothoraces. These injuries were attributed to the explosive force of the late deploying airbag crushing him against the unyielding seat back. The intrusions also caused multiple fractures of the lower extremities.

The child was unconscious in the front seat, which was in maximal forward position. After airlift to a trauma center, she was found to have seatbelt abrasions of her right neck and shoulder and her left hip, abrasions and contusions of her legs, and a left-sided laceration of the scalp. A CT revealed a depressed left parietal skull fracture and contusions and edema of the brain.

Unraveling the mechanism of the head injury required understanding a complicated series of failures of safety features in the Sebring. A paralegal noticed that the scar on the girl's head matched the configuration of the airbag door's corner. The pathologist superimposed an exemplar door in the scan to confirm the pattern.

The radiologist obtained a 3-D reconstruction of the calvaria from the original CT data. This showed a long, narrow depressed fracture suggesting impact on or from a dull rounded edge such as the airbag door which, on review of photos, had a bent corner facing the passenger's right. A luminol test showed blood on that corner of the door.

But how was the head juxtaposed to the door? The engineers remembered that failures of the latching system on the front seat rails of the Sebring had prompted a recall. This failure was confirmed in the involved vehicle. At impact the vehicle was twisted to the left, but the momentum of the child was directly forward, rotated her upper body clockwise and downward. A Canadian Transport test film had shown a delayed opening of both airbag doors of the Sebring. Thus, at an unfortunate millisecond in time, her accelerated left parietal area collided with the explosive force of the opening airbag door.

Follow-up medical evaluations predict permanent lower extremity disabilities and limited mental capacity at the 8-year-old level. A civil suit was filed for wrongful death, personal injury, and product liability.

This case emphasizes the value of assembly of multidisciplinary groups of forensic scientists in the analysis of complex events.

**Accident Investigation, Airbag, Safety System Failure**

## D18 A Cross-Sectional Study Road Traffic Fatalities and Vehicular Homicide Investigation Practices in Denmark for 2000-2004

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After attending this presentation, attendees will be acquainted with road traffic fatalities and vehicular homicide investigation practices in Denmark.

This presentation will impact the forensic science community by examining vehicular homicide investigation practices in Denmark, as the results indicate a number of limitations to these practices.

Unlike the relatively universally uniform criteria for criminal prosecutions associated with deaths resulting from violent assaults, the criteria for when to prosecute for vehicular homicide in a traffic crash fatality is quite variable between countries. Some of this variability is likely due to the fact that there are no widely accepted standards for what constitutes a comprehensive investigation of a potential vehicular homicide case. Police and medicolegal investigation practices of such cases in the Aarhus, Denmark Police District over a five year period were evaluated in order to assess the consistency of various practices used to investigate traffic fatalities.

Police investigation reports were obtained for all road traffic fatalities for the years 2000 to 2004 (inclusive) in Aarhus Police District, Denmark, an area with a population of approximately 333,000 people. A total of 81 crashes were found, with 209 individuals involved comprising 92 deaths, 61 injuries, and 56 uninjured people. Data concerning the circumstances of each crash were gathered along with information relating to the judicial course of each case, including prosecution, conviction, and sentence. Additional information from the autopsy report was correlated with the police investigation findings when an autopsy had been performed. The data were pooled and described.

Postmortem examination was performed in 17 of the 92 decedents (18%). Analysis for blood alcohol was performed in 55 (60%) of decedents, of whom 20 of the 55 (36%) were positive, and 17 of 20 positives (85%) had a BAC > 50 mg/dl. Toxicological investigation for prescription narcotics and the most common illicit drugs (i.e., cannabis and amphetamine) was performed in five (5%) of decedents, of which two (40%) were positive. There were a total of 80 surviving drivers, 42 of whom (53%) were tested for alcohol and one was tested for drugs/medicine. Amongst the surviving drivers the police investigation resulted in 33 (41%) cases of potential culpability. Of these 33 investigated drivers, 22 (67%) were tested for alcohol, with only one positive result (5%) and one was tested for drugs, with a negative result.

A total of 28 of the 33 potential offenders were charged with one or more violation, whereas five of the investigated potential offenders were not charged at all. Twenty-six of the 33 were charged with manslaughter under Danish law (§241). Of the remaining 47 drivers who the police investigation did not reveal potential culpability, 20 (43%) were tested for alcohol, and one of the 20 (5%) was positive. Of the 92 decedents, 61 were drivers, and 41 of these (67%) were tested for alcohol, with 12 positive results (29%).

Postmortem examination was poorly correlated with fatalities in which there was a manslaughter charge; of the 26 cases where there had been such a charge, only three autopsies were performed, yielding a rate of comprehensive medicolegal death investigation in criminal traffic crash death cases of 12%. In contrast, in the 54 cases in which a driver survived but was not charged with manslaughter, there were a total of 12 postmortem examinations, yielding a rate of comprehensive medicolegal death investigation of the cases where the police did not reveal any culpability that was almost double that of the cases charged with manslaughter (22%). Although the number of fatalities in the present study was relatively small, the population represented by the Aarhus Police District was considered to be representative of Danish practices in general, as Aarhus is the second largest police district in Denmark, surpassed only by Copenhagen. The results of the present study raise a number of questions concerning criminal investigation of Danish traffic crash fatalities. In contrast with common practice in the U.S., in which the most common reason for a criminal charge in a traffic fatality is intoxication of the offending driver, a large proportion of Danish drivers (one in three) were charged with a serious crime when alcohol presence was present in only one of the investigated surviving drivers, and autopsy was performed in less than one in five decedents. It appears that there is a lack of a standardized protocol for the investigation of potential criminal traffic crash fatalities in Denmark.

Further study is needed to determine if the results from Aarhus are consistent throughout Denmark. If this is found to be the case, a reappraisal of Danish practices concerning investigation of traffic crash homicides is warranted.

**Vehicular Homicide, Postmortem, Toxicology**

## D19 Trends in Suicide in Geneva, Switzerland: 1983 - 2007

*Romano La Harpe, MD\*, Institut Universitaire de Medecine Legale, Unite de Medecine Forensique, 9 Av de Champel (CMU), CH - 1211, Geneva, PQ 1206, SWITZERLAND*

After attending this presentation, attendees will understand trends in suicide in Geneva during the last quarter of century.

This presentation will impact the forensic community by identifying the following: quantifying the lethality of suicide methods used in Geneva, Switzerland, over a period of twenty-five years, and examining method-specific case fatality by age, gender, religious confession, marital status, dates, suicide method, and co-morbidities (alcohol and other illicit drugs use and mental illness).

**Methods:** A review of all autopsies conducted at the forensic medicine facility from the year 1983 to the year 2007. All the cases of suicide in Geneva, a little city of 400,000 inhabitants, go through the Institut Universitaire de Medicine Legale.

**Results:** During this period, there were 2007 documented cases of suicide; this number represents an average of 80 cases per year at a rate of 16.72 per 100,000 inhabitants. Of these deaths, 62% occurred in males (n = 1,243) and 38% in females (n = 764), for a ratio of 1.6:1.

The methods used were in decreasing order: jumping (22.5%), hanging (20%), firearm (19%), poisoning (15.5%), drowning (13%), CO (2.5%), cutting (2%), and others (5.5%: mainly throwing themselves in front of a train).

The most common methods of suicide among men were gunshot (27%), hanging (24%), and jumping (18%). In females, the most common methods were jumping (30%), poisoning (24.5%), and drowning (18%).

The most common method of suicide among men was firearm in all age groups, whereas in females in the under 25 age group – jumping, poisoning among adult age, and jumping again in the 65 and over age group. Gunshot was the most common method among unmarried, divorced, and widowed men, while hanging was the most common among married men. Jumping from heights was the most common method among unmarried, divorced, and widowed women, while poisoning was the most common among married women.

A significant change was not seen with the changes of season. The rate of elderly people who committed suicide was considered high, but it was very low for young people.

Finally, co-morbidity like depression, illness, alcohol, and other illicit drugs use were analyzed.

**Conclusion:** Despite the rate of suicide in Geneva being quite high, this rate has remained stable during the last quarter of century and it is especially low for young people.

**Suicide, Death, Geneva**

## D20 Custody-Related, Excited Delirium Deaths Following Intermediate Weapons Use in Ontario

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After attending this presentation, attendees will have an understanding of the Ontario experience with a review of three Excited Delirium (ED) in custody deaths that were investigated and subsequently examined in public inquests. In all cases intermediate weapons (either baton or conducted energy device (CED)) were deployed on the subject during apprehension by police. The inquests recommended legislative changes to allow CED access to all front line officers.

This presentation will impact the forensic community by examining the relationship between the intermediate weapons and the cause of death, highlighting how concerns regarding the often-controversial use of CEDs are likely not justified, while a non-controversial intermediate weapon may be lethal.

The Province of Ontario, population of 13.5 million, has a medical coroners' system. Under the Coroners Act, all deaths that occur while a subject is in police custody must be investigated and then examined at inquest, a public hearing that assures citizens that the circumstances of the death of no one of its members will be overlooked, concealed, or ignored. Three cases of individuals with Excited Delirium (ED) that evolved into custody deaths occurred in Ontario between August 2000 and July 2004. In all cases, there was a violent, prolonged struggle between the subject and officers before sufficient restraint and arrest could be affected.

The first case involved a male, age 55, with a long history of bipolar disorder and numerous hospital admissions for psychosis. Police were dispatched to a convenience store for an "unwanted guest" causing a disturbance. On their arrival, officers found the male to be agitated, but compliant. While obtaining routine information from him, he suddenly struck out at one officer. A violent struggle ensued as officers attempted to take control of him. In an attempt to curtail his flailing and kicking, he was forcefully struck multiple times by an extendible metal baton, but

seemed to be impervious to pain and exhibited extraordinary strength. Four officers eventually succeeded in restraining him in handcuffs in the prone position. Within seconds he went vital signs absent (VSA) and could not be resuscitated. The forensic pathologist found cause of death due to fat embolism complicating multiple blunt force soft tissue injuries. At inquest, the jury concluded the underlying cause to be Excited Delirium complicating Bipolar Disorder, and amongst its recommendations suggested that the prolonged struggle might have been avoided or shortened had officers been able to deploy a CED.

The second case involved a male, age 33, with a history of crack cocaine abuse and multiple prior episodes of ED. Police were summoned because of aggressive and violent behavior, and during prolonged attempts to apprehend him, used pepper spray with no apparent effect, and eventually three drive-stun mode CED deployments. The struggle lasted several more minutes before he was successfully restrained in wrist and ankle cuffs, then transported for medical assessment. At the hospital, he struggled again for several minutes before becoming VSA. Postmortem examination found no anatomic cause of death, with cocaine levels suggestive of binge use and consistent with those found to cause ED. The inquest jury found cause of death to be Cocaine-induced Excited Delirium, with prolonged struggle followed by restraint a significant contributing factor. They recommended that front line officers be authorized for CED use in the expectation that it might shorten the time to successful apprehension.

The third case involved another male, age 29, with a history of cocaine abuse and violent behavior. Police entered into a violent struggle with him when he tried to resist arrest. After several minutes, a tactical officer arrived and delivered a two-second long drive-stun mode CED deployment to his back. As with the previous case, the struggle to restrain the subject continued for several more minutes before he was successfully subdued in a hog-tied position. Shortly thereafter he went VSA. The pathologist attributed cause of death to Restraint Asphyxia, due to Cocaine-induced Excited Delirium. The inquest jury agreed with this conclusion, and again recommended that front line officers be authorized to use CEDs.

These three custody deaths are typical of cases of Excited Delirium, where subjects exhibit aggressive behavior, super-human strength, insensitivity to pain, and ineffectiveness of pepper spray during prolonged struggles with police officers. "Less lethal" use of force options, such as the extendable baton, may cause injuries that lead to death, while experiences to date in Ontario has shown no direct link between CED use and serious injury or death. Inquest juries have consistently recommended expansion of CED use to include front line officers, expecting that early deployment in cases of Excited Delirium will lead to faster control and apprehension and prevent deaths that result following prolonged struggles and prone restraint.

**Excited Delirium, Intermediate Weapons, Custody**

## D21    Suicide by an Unusual Improvised Firearm

*Marc A. Krouse, MD\*, Tarrant County Medical Examiner's Office, 200 Feliks Gwozdz Place, Fort Worth, TX 76104-4919*

After attending this presentation, attendees will increase awareness of the spectrum of unusual items that may be employed as an improvised firearm.

This presentation will impact the forensic community by expanding the knowledge of types of items that may be employed as improvised firearms. This knowledge is of importance to police, forensic scene investigators, forensic pathologists, and firearms examiners.

A 38-year-old male was found dead in his automobile in a parking area adjacent to a vehicle salvage business. A soot-covered defect was found in his shirt with an underlying gunshot wound that appeared as a contact entry wound. A wrench socket covered with tape and clamped in vise-grip pliers was found on the floorboard of the car near his left foot. A fired semi-automatic handgun cartridge was secured in one end of the socket. A claw hammer was also found in the front floorboard of the vehicle.

At autopsy, the subject had an entry gunshot wound over the precordium of his chest. Its appearance was consistent with a contact entry wound through clothing and a corresponding defect was found in the shirt. The "muzzle" imprint surrounding the wound and soot deposit had a hexagonal configuration. The wound track passed through the heart, diaphragm, and stomach, and deviated downward at the posterior left eleventh rib. A large caliber jacketed projectile was recovered from soft tissues of the lower left back; there were no visible land markings on the slightly distorted projectile.

The improvised weapon, with fired cartridge case in place, and the recovered projectile were inspected. The cartridge had been secured in the base of the socket by metal wire inserted in the extractor groove and twisted on each side. This prevented the rimless cartridge from falling into the socket and held it in place firmly enough for more than one blow by the hammer to discharge the round. The projectile itself bore varying striations from interaction with the sides of the socket.

This case presentation is intended to inform medical examiners, death investigators, police investigators, and firearms examiners of the unusual features of unique and improvised firearms.

**Improvised Firearm, Standard Tools, Suicide**

## D22    Corresponding With "The Happy Face Killer" – A Case Study

*Lyndsie N. Schantz, BS\*, 700 Forbes Avenue, Apartment 1816, Pittsburgh, PA 15219*

After attending this presentation, attendees will be introduced to a unique opportunity where Duquesne University Forensic Science and Law students corresponded with a serial killer for over a year.

This presentation will impact the forensic community by examining the psyche of a murderer as well as discuss the errors made by law enforcement and how these miscalculations affected this specific case.

The goal of this presentation is to introduce the members of the forensic science community to a unique opportunity where Duquesne University Forensic Science and Law students corresponded with a serial killer for over a year. Criminal Investigations, a two semester upperclassman course led by Former Pittsburgh Police Commander Ronald Freeman sought to initiate correspondence with the "Happy Face" Serial Killer, Keith Hunter Jesperson, in order to gain the perspective from the polar side of law enforcement. Jesperson is the subject of several documentaries and the book, I: The Creation of a Serial Killer by Jack Olsen. This presentation will impact the forensic community by examining the psyche of a murderer as well as discuss the errors made by law enforcement and how these miscalculations affected this specific case.

Keith Hunter Jesperson is accused of murdering eight women by means of strangulation. He committed these murders in numerous jurisdictions across five states. While he has confessed to all of them, he has not yet been prosecuted for every homicide. Jesperson's familiarity with destroying the identification of his victims allowed him to elude arrest for years. However, the real twist of events came about when two other people were convicted of his first murder. Wrongful convictions have become a topic of great interest in the past few years. Jesperson himself was not the victim of a wrongful incarceration rather two other individuals who claimed to have knowledge of the murder were convicted for the murder. They were imprisoned for years until Jesperson was approached by the police regarding the death of his girlfriend, his final victim. During this period, Jesperson attempted suicide twice and then was arrested for killing his girlfriend. During one

of the suicide attempts, he left a letter to his brother chronicling his murders. With his brother releasing this information to the police, Jesperson finally confessed to the killings and subsequently fought for the release of the two individuals who were wrongfully convicted.

The impetus for the project came about when the course instructor read I: The Creation of a Serial Killer, which is a narrative of Jesperson's life. Mr. Freeman presented his idea of corresponding with Jesperson to his Investigations class. Three students readily volunteered to initiate the correspondence with the killer. There was a steady flow of letters exchanged on the order of once a week. Immediately, students perceived Jesperson's need to control in his communication with the class. In addition to the letters, a one hour phone conversation allowed the class to hear the voice of a murderer.

Although it was the wrongful conviction that sparked the interest of the class, the correspondence with Jesperson taught the class so much more, including insight into the dark nature of humanity. The individuals in the class were exposed to the criminal mindset as well as the point of view of law enforcement due to the professor's extensive knowledge from over three decades of working in the homicide division. This was an incredibly unique opportunity afforded to a group of senior Forensic Science and Law majors. Not only was this project a great learning experience, but it can be used in the justice system to help convict Jesperson of a prior murder.

**Serial Killer, Wrongful Conviction, Criminal Mindset**

## D23 Effective Use of the Multidisciplinary Approach Critical to Solving Contemporary Violent Crime

*David J. Zeliff, MFS\*, U.S. Army Criminal Investigation Command, 6010 6th Street, Fort Belvoir, VA 22060; and Michael J. Bosse, MFS\*, 3411 Mercedes Drive NE, Lacey, WA 98516*

After attending this presentation, attendees will understand the increasing sophistication and planning of violent crime by contemporary criminals, the impact on violent crimes due to the explosion of forensic science knowledge in mainstream media, the necessity of employing a multidisciplinary scientific approach when conducting violent crime investigation, and the enduring and critical role of "old-fashioned" detective work, when investigating contemporary violent crime.

This presentation will impact the forensic community by demonstrating that the complexity, motivations for, and planning of contemporary violent crimes necessitate adherence to time-honored crime scene processing and investigative techniques bolstered by effectively using the wide spectrum of scientific disciplines within the forensic community to effectively resolve contemporary violent crime.

The explosion of forensic crime dramas and true-life documentaries in the main stream media, and the parallel increase in available academic forensic education, has forever impacted the art of criminal investigation. Savvy criminals can easily learn through a wide variety of sources how to circumvent traditional crime fighting strategies, thwart traditional crime scene processing techniques, and mask or obliterate various types of physical evidence traditionally associated with interpersonal violent crimes. The authors have seen, over the course of their combined five decades of criminal investigative experience, a significant shift in the time and effort spent by perpetrators in altering, and sometimes thoroughly staging, violent crime scenes. These activities occur to avoid forensic detection, in even the most emotion, anger, and drug-fueled violent crimes.

Case studies will be presented which illustrate how knowledge of forensic science affected the planning and execution of violent crimes, and the efforts at crime scene manipulation by perpetrators in the aftermath. The case studies will demonstrate how leveraging the multidisciplinary approach in processing the crime scene, incorporating the forensic pathology and laboratory findings, and combining the results with traditional stalwart investigative methods resulted in identification of the perpetrators. The case studies anecdotally support the belief of the authors that contemporary violent criminals demonstrate an increasing forensic sophistication in their crimes.

**Crime Scene, Multidisciplinary, Investigation**

## D24 From 1997 to 2007: Modified Approach to Sexual Assault in Our Experience

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After attending this presentation, attendees will understand the medico-legal findings in a population of sexual assault cases assessed in Palermo in the years 1997-2007.

This presentation will impact the forensic community by demonstrating the role of adequate training of health professionals in this field of forensic application. An interdisciplinary approach has a strong impact with regard to sexual abuse and "gender" violence (i.e., against children and women), how victims survey their situation, and the new approach of the Italian legislation which offers the widest support to victims.

An essential aspect of sexual violence is represented by the condition of the victim. Another aspect of sexual violence is if the victim can make an informed decision with regard to what has happened to them and the different proposals offered to them.

Sexual abuse can be considered even in cases where the victim is never physically touched. An example of this would be where a victim has viewed an act(s) of a sexual nature or made to listen to conversations with a sexual content. These are classified as sexual abuse cases because the victim has seen or heard sexual content that is not age appropriate or because of their relationship with the abuser.

Furthermore, intra-family sexual abuse produces, in principle, the most serious effects, even when it is compared to abuse that has occurred outside of the family.

According to Italian law (act 609 bis of law 66/1996), anyone who uses violence, threats, or abuse of authority to force individuals to perform or undergo sexual acts is punished with imprisonment anywhere from five to ten years.

If the sexual act is committed on a person who is mentality or physically impaired or challenged, or if the perpetrator blames another for their acts, they too will receive imprisonment anywhere from five to ten years.

Act 609 also includes language that states there is the penalty of imprisonment anywhere from six to twelve years, if the sexual act is committed to: (1) Any one under the age of fourteen, (2) Use of weapons, alcohol, narcotics, drugs or other substances that can seriously damage the health of the victim, (3) Impersonation of a public official or someone with official authority, (4) Limits to the personal freedom of the victim, and/or (5) If the victim is under the age of sixteen and the perpetrator is a parent, adoptive parent or a guardian. If the victim is

under the age of ten, the penalty of imprisonment is increased to seven to fourteen years.

Sexual abuse can produce many kinds of psychological problems, which are subjective in nature. Some of these subjective responses are influenced by age of the victim, duration of the abuse, the presence or absence of penetration, the use of violence, personal characteristics of the victim, concurring psychological problems, if the victim can share their experience of abuse with someone, emotional support, and other life experiences. These life experiences may worsen or help the victim gradually overcome the abuse.

In 2005, a team was assembled that includes forensic physicians, gynecologists, surgeons, and psychiatrists who can examine an assault victim. The legal authorities or the victim can request an examination. The examination consists of a medical history and the clinical examination follows adopted standardized procedures. The victim is also given information with regard to their personal protection. The team also collaborates with non-profit agencies that focus on human rights and are authorized by a European Council.

During this period about 100 victims of sexual assault were examined, recording demographic information (age, gender, time elapsed before consultation), circumstances about sexual assault (date, place, assailant's identity when known, frequency of the assault), type of assault (penetration, non-penetration).

The circumstances of the assault were based on the victims' and any witnesses' statements.

The medico-legal outcomes revealed that of 100 victims examined, 12 were males and 88 females; 35 of these victims were children less than ten years of age; 31 victims were between the ages of 10 and 14 years old; and 34 were older than 14 years of age.

The assailants were, for the most part, fathers. In less than one-third of the cases were there signs of sexual violence (bruising, excoriations, abrasions).

In this presentation, research data obtained which relates to the social, cultural, and legal outcomes of medico-legal evaluation in court systems will be analyzed.

**Sexual Abuse, Medico Legal Examination, Legal Outcome**

## D25    Imposters:  Physical Findings That Can Be Mistaken for Sexual Assault Injuries

*Patricia M. Speck, DNSc\*, 1740 Overton Park Avenue, Memphis, TN 38112; and Diana Faugno, MSN, 1351 Heritage Court, Escondido, CA 92027*

After attending this presentation, attendees will learn how to distinguish between normal conditions, diseases, and injury in the sexual assault victim and apply new knowledge to case studies.

This presentation will impact the forensic community by reviewing existing information and highlighting the importance of distinguishing between normal findings and conditions that are made worse because of injury. Health care providers will use this information for differential diagnosis.

The interpretation of the injury following sexual assault has been challenged in recent literature. Noted physical findings, in particular bruising patterns, may be confused with intentional injury, when they are in fact unintentional. Literature has revealed that injury may occur in consensual relationships and a body of literature exists that describes conditions that will be made worse with penetration that is not forced. The cyclic nature of genital tissue response to estrogen has been established and mid-range theories have been promulgated that explain some injury in the pre-menarchal and post-menopausal females. In addition, complex changes to genital tissue occur with conditions or infections that may or may not be sexually transmitted. Case studies will be presented that are complex and require decision making by the health

care provider charged with evaluating the victim of sexual assault. Tools that assign classifications will be introduced to compare and contrast their usefulness in determining whether or not the injury is from sexual assault.

**Non-Specific Injury, Blunt Trauma, Specific Injury**

## D26    Forensic Nurses on Crime Scene:  The Southern Italian Experience

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The goal of this presentation is to provide an overview of the development of forensic nursing in Southern Italy and to illustrate the unique contributions of the professional nurse in death investigation.

This presentation will impact the forensic community by demonstrating how nurses are utilized within the Office of the Medical Examiner of the University of Bari. The use of forensic nurses has positively impacted the forensic science community and demonstrates the unique contributions of the professional nurse in solving cases.

The University of Bari has chosen to give major emphasis to forensic nursing in the advanced educational degree (i.e., Magisterial Bachelor Degree). The educational program addresses forensic topics such as elder and child abuse, domestic violence, mass disasters, evidence collection and preservation, and death investigation. A Master's program on postmortem findings and crime scene investigation provides additional information and advanced clinical training in the field. Four typical examples of forensic nursing activities in Bari will be presented.

**Case #1 - Multiple Self-Inflicted Incised and Stab Wounds**: In September 2007, a 48-year-old man was found dead in his summer residence by his wife and daughter. Police investigators, a medical examiner, and a forensic nurse investigated the crime scene. The man's body was on the floor of bedroom; his shirt was unbuttoned and soiled with blood. There was also blood on the bedroom floor and on the bed. A kitchen knife was found near the body and there was a suicide note on the bedside. The cause of death was 36 incised and stab wounds. In this case, a forensic nurse recorded body and environmental temperatures, collected evidence while preserving the chain of custody, and took on-scene photographs. Upon order of a prosecutor, a few days later, the nurse interviewed relatives and friends of the victim to reconstruct the last moments before death in order to understand the manner of death.

**Case #2 – Starvation Due to Religious Delirium:** In July 2007, the bodies of two elderly sisters were found mummified on chairs within their country home. A third sister was found alive, but with mental confusion. Mummified carcasses of cats and dogs were found close to the bodies. There were small containers with white powder that was later identified to be sodium carbonate ($Na_2CO_3$). Procedures to solve the case involved the contributions of different forensic specialists including a pathologist, nurse, entomologist, criminologist, toxicologist, and a veterinarian to define the time since death, the cause and manner of death, and to obtain a psychiatric profile of the surviving sister. The role of forensic nurse was important in noting and collecting any evidence at the crime scene: mapping the positions of the bodies,

documenting entomological findings, searching for unknown substances closest to the bodies, etc. The nurse discovered a secret diary of the surviving sister where she noted daily events during last three years. In this diary there were many attached receipts, checks, and bills. All of this evidence was useful in understanding what happened in the house and the reasons that the remaining live sister continued to reside among the mummified bodies.

Case #3 – Work-Related Death: In February 2008, four workers died suddenly from inhaling hydrogen sulfide ($H_2S$) while washing a road-tanker at their workplace. One died 24 hours later in a public hospital. The forensic nurse interviewed the other workers on the scene, documented evidence about substances that the tanker was transporting, and went to the hospital to review clinical documentation of the survivors a day later.

Case #4 – Anthropological Excavation: In August 2007, human remains were discovered by a forester during a search of the country surrounding Miglionico (Southern Italy). There were 286 bone fragments excavated that had been buried. The forensic nurse with a special background on skeletal remains, assisted with verification of personal identity during all phases of the excavation procedures and examination of each fragment. His contributions were particularly effective in the collection and packing of all the evidence that had to be sent to the laboratory.

Conclusion: Forensic Nursing is a new career opportunity in the Office of Medical Examiner of Bari (Southern Italy). The Office oversees the majority of forensic cases associated with 48 towns located in the Region (Apulia) with a total population of about 1.5 million. Special investigations are submitted from other southern regions (Calabria, Basilicata, Campania) when a specialized forensic team is required in solving a case. The forensic nurse is an effective member of the forensic team.

**Forensic Nursing, Crime Scene, Death Investigation**

# D27 The Council of Forensic Medicine: A Tragedy or Good Luck for Turkey?

*Nevzat Alkan, MD\*, Istanbul Tip Fakultesi, Adli Tip Anabilim Dali, Capa, Istanbul, 34390, TURKEY*

After attending this presentation, attendees will understand the structure of the council of forensic medicine in Turkey and will have discussed if can it be a model for other countries.

This presentation will impact the forensic community by presenting knowledge about Turkey's forensic medical system.

Expertise in forensic sciences is an important tool for law processing in all countries. Forensic examination is needed for an objective solution in majority of cases.

Ataturk and his associates founded the Turkish Republic in 1923. This new Republic encouraged different ways of thinking and higher education, which all lead to developments that have become standards in the modern world. One area that benefited from these developments was the modernization of the legal arena. The Council of Forensic Medicine, charged under the Ministry of Justice in 1923, was but one of the institutions formed in the young Republic.

The Council of Forensic Medicine is an expert organization located in 50 out of the 81 cities in Turkey. The Council's headquarter is in Istanbul. The Council's framework includes an assortment of medical disciplines.

Located in the Council's headquarter, are six specialized committees and six specialized departments. The duty of the 1st Specialized Committee is death investigation, while that of the 2nd is to report on intentional laceration; 3rd on occupational diseases, malpractice cases, and deferment of detention due to old age or illness; 4th on forensic psychiatry; 5th on cases of poisoning and allergy; and 6th on domestic violence, sexual assault, and child abuse. Among the six Specialized Departments, the Morgue has the duty to perform autopsies, while the Chemistry Department makes toxicologic and narcotic analysis in body fluids. The Biology Department performs paternity testing and DNA applications; the Physics Department's duty is to perform document examination, trace analysis and ballistic examination; the Traffic Department investigates the forensic cases that have resulted from traffic accidents. The Psychiatry Department has an Observation Unit, and after a three week evaluation, passes its final opinion on the patients to the 4th Specialized Committee.

Under the Ministry of the Interior, there are four gendarme and ten police criminal laboratories located in various city centers throughout Turkey. Included in these laboratories are various forensic departments. At this time, there are no advanced private forensic laboratories.

Forty-one out of the forty-five medical facilities that are housed in the seventy-five universities in Turkey have forensic medical departments. These facilities have inadequate infrastructure and personnel. This limits routine applications. Courts often require expertise, which comes from various non-forensic institutions. These institutions are not well organized and lack modern technology. Therefore, the most of the workload is given to the Council of Forensic Medicine in all parts of Turkey.

In this presentation, information on the structure of the forensic sciences and forensic medicine in Turkey, as well as the expertise areas, is given. The different aspects of the topic "The Council of Forensic Medicine: A tragedy or good luck for Turkey? Can it be a model for other countries?" is discussed.

**The Council of Forensic Medicine, Turkey, Forensic Medicine**

# D28 Science for Justice and Health: The UNODC's New Forensic Work Program

*Justice N. Tettey, PhD\*, United Nations Office on Drugs and Crime, Vienna International Centre, Vienna, A-1400, AUSTRIA*

After attending this presentation, attendees will learn about the new forensic work program of the United Nation's Office on Drugs and Crime (UNODC) whose goal is to increase the quality of forensic science services worldwide from the crime scene to the forensic laboratory.

This presentation will impact the forensic community by demonstrating the importance of forensic science organizations in the delivery strategy of the UNODC's forensic services worldwide.

In 2007, the Laboratory and Scientific Section of UNODC embarked on a forensic work program which aimed at increasing the worldwide availability of quality forensic services from *the crime scene to the forensic laboratory*. This presentation looks at the relevance of the program to the organization's strategy on drugs and crime. Also, the mechanisms of support in forensics such as the international collaborative exercise and worldwide collaborations and partnerships are discussed and the presentation concludes by looking at the current priorities of the work program and examining the role of forensic science organizations in achieving the UNODC's objective of Security and Justice for all.

**United-Nations, Forensic, Crime**

## D29 The Preparation of a United Nations Manual on Crime Scene Investigation for First Responders and Policy Makers in Developing Nations

*Peter R. De Forest, DCrim\*, John Jay College/CUNY, 445 W 59th Street, New York, NY 10019; and Justice N. Tettey, PhD, United Nations Office on Drugs and Crime, Laboratory and Scientific Section, Vienna International Centre, Vienna, A-1400, AUSTRIA*

After attending this presentation, attendees will gain an awareness of the role of the United Nations in assisting developing nations in establishing forensic science systems.

This presentation will impact the forensic community by showing how the manual will guide governments in developing nations in setting up scientifically based systems for the investigation of crime and event scene.

This presentation will describe a concise manual on crime or event scene investigation being developed by the United Nations Office on Drugs and Crime (UNODC). The target audience for the manual is first responders and policy makers. The working title of the manual is: *Crime Scene and Physical Evidence Awareness for Non-Forensic Personnel*. The manual was designed to assist governments in developing nations to establish and improve the capabilities of first responders and promote a scientifically based crime scene investigation. The preparation and publication of the manual is part of a larger initiative of the UNODC in assisting developing nations with the establishment of forensic science systems to foster its dual goals of security and justice in the developing world.

**United Nations, Crime Scene Investigation, Developing Nations**

## D30 Death Caused by Trash Collector Crushing: A Case Report

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After attending this presentation, attendees will learn about a case, which had limited exposure in the form of literature. In the presented case, the victim had initial blunt trauma to the chest, which evolved into open trauma, and resulted in a death due to a trash collector's actions.

This presentation will impact the forensic community by explaining, that unlike "traditional" blunt force trauma, a body that has had an extreme amount of force placed on it from an "externalized" source makes for a novel mode of death and detection of the body.

The victim was a 71-year-old Caucasian male whose corpse was found in a public landfill. When the corpse was discovered it was in an advanced state of putrefaction. The victim had the habit of looking through trash dumpsters and it appears that the victim lost his balance and fell inside the dumpster. Once the victim was inside the dumpster, the lid closed. The victim could not open the dumpster's locked lid, as it could only be opened from the outside by way of a foot pedal, due to a unique spring mechanism.

The victim remained in the dumpster for at least a few hours, possibly having been stunned by the smell of the garbage, and was crushed/compressed by the trash vehicle that collected the trash. The now compressed victim, inside the dumpster, was taken to the public municipal landfill the next day where the dumpster was emptied.

The victim, who sustained a high degree of compression, was crushed. There was closed trauma to the chest and due to the compaction forces lead to complex injuries. The injuries that were sustained by the victim were evisceration of the thoracic and abdominal organs, crushing to the chest, spine and bones, migration of the thoracic organs from its diaphragmatic breach, explosive injuries to multiple organs, and decay of parenchymatous organs which resulted in the virtual instantaneous death of the victim.

The autopsy report of this victim will be presented. Photographic material will show the presence of large hemorrhagic infiltrations, fractures and galeal bleeding of the victim's head and will confirm that the victim was alive when he fell into the dumpster, and that his death could not have been a result of anything else, except being crushed/compressed to death in the dumpster.

**Trash Collector, Blunt Trauma Chest, Crushing**

## D31 A Review of Asphyxia Cases in the Lincoln, Nebraska Area From April 2003 to July 2006

*Casey C. Anderson, BA\*, University of South Florida, 4202 East Fowler Avenue, SOC 21E, Tampa, FL 33612*

After attending this presentation, attendees will be provided an overview of: the epidemiology of asphyxia deaths in the Lincoln, Nebraska area, the importance of compiling a regional database among this type of death to aid death investigations, and the characteristic injury patterns from different types of asphyxia deaths.

This presentation will impact the forensic science community by using this regional compilation method to compare and help predict the epidemiology and demography of underlying factors affecting asphyxia deaths, increasing awareness of the different types of asphyxia cases, uncover risk factors in reference to SIDS (Sudden Infant Death Syndrome) deaths, as well as observing the epidemiological and demographic differences of asphyxia deaths between regions. This study can be utilized on a large scale for discovering regional differences to better understand different asphyxia deaths. The injury patterns will also allow death investigators to compare injuries found on a body of an asphyxiated decedent to injuries established from different causes of death. This will give investigators a cross-reference database to confirm findings in difficult or suspicious cases and possibly lead to conclusions that are more accurate.

Data was collected from autopsy reports and investigation supplements provided by the city of Lincoln's forensic pathologist, ranging from the dates April 2003 to July 2006. The 68 identified cases were then compiled and statistical tests were performed using a computer program in order to uncover the frequencies of different variables. The variables tested were: age, sex, ancestry, cause of death, manner of death, location, drug use, and injuries sustained related to the death. The seven types of asphyxia cases analyzed are: Sudden Infant Death Syndrome (SIDS), drowning, hanging, carbon monoxide poisoning, suffocation, and positional asphyxia. The collection of data from the forensic pathologists' records give an accurate depiction of what death investigators will find at the time a body is discovered.

The results illustrate most asphyxia deaths in the Lincoln area were predominately comprised of Caucasian males with a mean age of death at 27.7, and large drug usage. Natural death, more specifically SIDS, case results showed the majority of the decedents' deaths occurred in a crib, and half of the total number were ill. Cyanosis was also found to be present in a significant portion of the cases when the decedent was discovered. Two types of accidental deaths, suffocation and drowning, were established. Accidental suffocation cases showed a significant portion of deaths occurred in a bed with a large percentage of the victims

being children. Accidental drowning victims were equally found in locations either in or around the immediate home, or a lake, and a significant amount of the victims had some form of cranial hemorrhage. Lastly, suicides were the most dominate manner of asphyxia, consisting of 35.29% of the total cases. The majority of suicidal hangings used an electrical cord, and an equal distribution of the cases used either an article of clothing or a rope as the next most common ligature choice. Neck abrasions from the ligatures were most commonly found, followed by contusions, then lacerations from the ligature. Cyanosis was found in a small percentage of the cases. Location data showed that the home was the most common place for a suicidal hanging to occur, followed by an outdoor location. An interesting injury pattern associated with suicidal hangings showed that those who used a nylon rope were the ones with a presence of petechiae, specifically in the larynx, trachea, and lungs. Lastly, suicidal carbon monoxide poisoning all occurred within the victim's car. Positional asphyxia cases were found to have inconclusive data because of the lack of a significant sample size.

According to the 2007 U.S. Census estimate, the demographics of the Lincoln, Nebraska area population leans towards a more dominate Caucasian population (89.25%), with 99.2 males to every 100 females, and the average age of residents being 31 (U.S. Census Bureau). These variables are examples of why regional databases need to be made, for they could change according to the demographic profile of the region. Death investigators could greatly profit from such a model, for similar cases could be cross-referenced and used to efficiently close a case.

**Asphyxia, Epidemiology, Regional Model**

## D32 Describing the Setup and the Functionality of a Medicojudiciary Unit for Sexually Assaulted Children in France

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After attending this presentation, attendees will be briefed on the characteristics of a medico-judiciary unit for sexually assaulted children. The unit gathers various competences in a same place and at the same time in order to optimize judiciary operations while protecting children's health.

This presentation will impact the forensic community by promoting and developing the concept of multidisciplinary units for sexually abused children.

The increase in sexual abuse allegations to children and the diversity of support agents involved has led to a general review of healthcare management.

It is clearly important to be able to coordinate the necessary judiciary interventions with the medical, social, and psychological support for the child. In order to protect the child and the prevention from any further trauma, the child should be cared for in a single place, where all the support agents could meet him or her. Such a unit has been created at the University Hospital of Angers. This multidisciplinary unit is called the Permanent Pediatric Centre for Children in Danger (Permanence d'Accueil Pédiatrique de l'Enfant en Danger).

This center was created in 2005. In this facility, 845 children have been seen (female: 66% and male: 34%). The average age, at which the medical examination took place, was 15 years of age, with the youngest victim at nine months of age and the eldest at 18 years of age. Sexual abuse was presumed to be suffered in 89% and for 11% of the cases physical violence was also suspected. The police are also involved in this structure. A nurse is present to receive the child/children and its

family. Investigators can use a special room to interview the children and then document the conversation. After the interviews, forensic examinations are performed by a forensic medical examiner in the same department and in a special room (with colposcope). The same nurse is present during the examination. Pediatricians can be called to give some medical advice or provide medical care.

The family and the children also meet with the social worker and, sometimes, a psychiatrist. This unit seems to be an appropriate answer, facilitating various competences while respecting particularities and the field limits of each support agent.

Characteristics and the particular requirements of the care of sexually assaulted children in this medico-judiciary unit will be explained.

**Sexual abuse, Child, Genital Examination**

## D33 Infrared Analysis of Human Remains

*Diane K. Williams, PhD*, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, the attendee will gain a better understanding of how infrared spectrometers may be used to detect human remains.

The presentation will impact the forensic community by providing an additional tool for the detection and location of human remains.

Human skin has been shown to possess reflectivity in the short-wave infrared (SWIR) region of the electromagnetic spectrum. As a result, a research study was designed to investigate the reflectivity of human skin in the (SWIR) region of the spectrum using a hand-held infrared spectrometer. The goal of the study was to use the infrared spectrometer to collect reflectivity data from the skin of a wide range of humans and from tree canopy to assess the feasibility of using a portable SWIR system to recover human remains. Prior to the collection of the skin reflectivity data, preliminary measurements were made on fabrics to determine instrument reproducibility. The results from the preliminary measurements suggest that the instrument is reproducible since the signal variation was less than five percent.

The reflectivity data was collected from the skin, hair, and bones of human remains in the short-wave region of the electromagnetic spectrum to determine the characteristic reflected wavelengths from each type of sample. The data was collected from the human remains housed at the Anthropological Research Facility at the University of Tennessee at Knoxville. The remains were in various stages of decomposition (ranging from one to four years), and all of the measurements were made in situ over a period of several days in order to account for variations in weather. Spectra was collected from both male and female remains. Multiple sample measurements were obtained in order to determine the mean and standard deviation of each sample type. Additionally, radiance measurements were taken from the ground through tree canopy to determine the amount of reflectivity that might be detected from a remote imaging camera. Finally, reflectivity data was collected from foliage and other environmental debris to determine wavelengths of possible interferences. The data was analyzed for consistency and principal component analysis of the data is continuing.

The initial analysis of the data revealed that there are unique and characteristic wavelengths in the SWIR region of the electromagnetic spectrum that will distinguish human skin from tree canopy. Additionally, the data analysis revealed that the spectra collected from human hair are reproducible and are not dependent on variables such as the sex of the individual or hair color. The complete data set will be discussed in the context of the spectral differences between human remains and tree canopy and the ways in which these differences may be used to aid in the search for human remains. Additionally, the relevance of the data with regard to the types of possible environmental conditions and/or interferences will be discussed. For example, a variety of tree leaves were analyzed to determine the effect of species on the analytical

signal in the SWIR region. An initial review of the data suggests that the technique shows promise for using a SWIR system for detecting human remains. Future research will focus on the determination of the key spectral parameters that will be useful for further field testing using a remote system for detection.

**Human Remains, Infrared Spectra, Remote Sensing**

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## D34 Hyperspectral Imaging of Post-Blast Explosive Residues

*Diane K. Williams, PhD, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135; and Kerri Lynn Moloughney, BS\*, Oak Ridge Institute of Science Education, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will gain a deeper understanding of the use of hyperspectral imaging systems to detect explosive residues.

This presentation will impact the forensic community by providing the explosives analyst with an additional tool to detect and visualize explosive residues on variety of substrates.

Hyperspectral imaging (HSI) allows for the conversion of spectra into image information, allowing visualization through a much wider range of wavelengths than is possible with other imaging methods. As compared to multispectral imaging, which has a single-digit order-of-magnitude wavelength range, HSI can record images over hundreds of wavelengths with very narrow bands. A highly specialized hyperspectral camera has previously been used to detect explosives on fabrics.[1] Based on these results, a study was initiated to detect and identify post-blast explosive residues using this hyperspectral system.

Post-blast residues present a different challenge from the previous work, due to various environmental factors. Debris, dirt, and human contact can all have an effect on the spectra of the residue. Control samples of explosives were provided by the FBI Explosives Unit and analyzed to determine the key wavelengths where spectral characteristics may be useful. Hyperspectral images were taken of samples obtained from an explosives demonstration. A range of explosives were detonated outdoors, and many of the samples obtained had dirt and debris on them. A variety of substrates was chosen to test, including plastic, wood, metal, concrete, fabric, paper, tape, and glass. All of the samples were collected immediately after the blast, placed into separate containers and appropriately labeled.

The data set represents three dimensions, two spatial and one spectral. Scans of wavelengths ranging from 400-950 nm collect a complete spectral profile for each pixel in the two dimensional image. The "data cube" constructed enables the user to determine the precise location on the image from which a particular spectrum was obtained. This spectral profile also allows visualization of chemical differences on the image itself. Using image processing software, specific spectral characteristics are isolated and illuminated during post-processing. The data will be presented in the context of both detection and visualization of the post-blast residues. A discussion of the reproducibility of the data will be included.

Based on the previous results obtained, it is hypothesized that the hyperspectral imaging system will provide the desired information regarding the key spectral wavelengths for visualization of the residues on a variety of substrates. A long-term study will follow the initial study to determine the effect of aging on the chemical signatures of the samples as well as a reproducibility study. Upon completion of the data set, these key spectral wavelengths will be used to develop a protocol for the detection and visualization of post-blast residues on a variety of substrates. The protocol will be particularly useful because hyperspectral imaging is a non-destructive analytical technique and allows for simultaneous detection and visualization, even when the residues are invisible to the human eye. Additionally, hyperspectral imaging cameras can be used for remote sensing; therefore, the research will be expanded to include the testing of the protocol for remote sensing capabilities.

**Reference:**

1 Williams, D.K., Ayub, H., "Detection of Explosives by Hyperspectral Imaging", 234th Meeting of the American Chemical Society, Boston, Massachusetts, August 19, 2007.

**Explosives, Hyperspectral Imaging, Remote Sensing**

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## D35 Age and Gender Differences in Suicide Trends in Puerto Rico: 1999-2007

*Enid J. Garcia-Rivera, MPH\*, Puerto Rico Department of Health, PO Box 70184, San Juan, 00985*

After attending this presentation, attendees will learn about demographics, age, and gender-specific differences in suicides in Puerto Rico from 1999 to 2007.

This presentation will impact the forensic community by showing the changes in suicide trends, and the age and gender specific differences provide forensic and public health officials important information for the identification of high risk populations and the development of targeted public health interventions.

Suicide is an important public health problem throughout the world. Approximately one million people committed suicide every year, with a global increase of 60% in the last 50 years. In 2004, suicide was the 15th leading cause of death in Puerto Rico and the third leading cause of death among males ages 15 to 29. With the purpose to further understand changes in the frequency and distribution of suicides in Puerto Rico we evaluated suicide trends by age, gender, method, and geographical distribution from 1999 to 2007.

Annual suicide data were obtained from all cases investigated at the Puerto Rico Institute of Forensic Sciences (PRIFS) from 1999 to 2007. PRIFS receives all suspected suicides from Puerto Rico for investigation. Descriptive statistics were used to characterize the study population. U.S. Census population estimates were also used as denominators in suicide rates calculations. Suicide mortality rates during the nine-year period were age-adjusted to the 2000 standard population for Puerto Rico and stratified by gender and age for analysis. The annual percent change (APC) from 1999 to 2007 in suicides rates was calculated.

From 1999 to 2007, the PRIFS analyzed 52,122 cases of which 2,792 (5%) were classified as suicides. The mean annual number of suicides was 310 per year. The age-adjusted suicide mortality rates ranged from 8.5 per 100,000 population in 1999 to 7.9 per 100,000 population in 2007. No significant changes on the suicide rates occurred during the study period (APC -0.35, no statistically significant). The mean suicide rate for males was seven times the rate for females (14.6 suicides per 100,000 population vs. 2.0 suicides per 100,000 population, respectively). Most suicides occurred in persons from 25 to 54 years (57%). However, the cumulative suicide mortality rate was highest in persons older than 75 years (28 suicides per 100,000 population) followed by persons aged 45 to 54 years (24 suicides per 100,000 population) in both genders.

Overall, the method most commonly used to commit suicide was hanging (64.5%), followed by fire arms (14.1%), solids or liquid poisoning (13.4%), jumping or falling (2.7%) and burning (2.7%). Gender-specific analysis showed that although hanging was the preferred method for both men (67.4%) and women (44.3%), the second most common method for men was fire arms (15.3%) vs. poisoning (32.1%) for women.

Higher suicides rates than the expected for the population (>7.9 cases per 100,000 population) were observed in several rural and low-income municipalities in Puerto Rico.

Although the rates of suicide in Puerto Rico have remained stable from 1999 to 2007, gender and age specific differences were identified. Understanding changes in suicide trends and the age and gender specific differences provides forensic and public health officials important information for the identification of high risk populations and the development of targeted public health interventions. In addition this presentation will impact the forensic community by presenting trends, and age and gender differences in a Hispanic community in the United States.

**Suicide, Trends, Hispanics**

## D36 Voice Stress Analysis: A Comparison of Layered Voice Analysis Instrumentation and Auditors' Judgments in Detecting Deception in a Field Setting

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After attending this presentation, attendees will learn if the truthfulness or deception of criminal suspects is detectable from audio-recorded interviews by instrumental or non-instrumental means.

This presentation will impact the forensic community by highlighting concerns about the use of voice stress analysis as a means of detecting deception for forensic and other purposes in the law enforcement community. In addition, what these findings suggest about the interpretation of research reports on voice stress analysis will be considered.

There are two points of information attendees should take from this presentation. First, it is possible to detect the "deception" of criminal suspects from audio-recorded interviews. Second, such auditory detection rates may exceed those obtainable with commercially available "voice stress analysis" devices. Attendees will learn that this latter point raises a concern regarding forensic and law enforcement use of such devices and of the value of some of the evidence advanced in favor of their use.

In this study, two highly experienced interviewers (serving as auditors) were provided with audio-recorded interviews (about 25 minutes in length) of 73 persons who were suspected of involvement in criminal events being investigated by a police agency. When evaluating the audio files each auditor rendered three decisions. First, after listening to the initial portion of each file, a decision of "truthful" or "deceptive" was made; this decision was based on the interviewee's responses in the first several minutes of the interview. Second, at the conclusion of the entire interview each auditor again rendered a decision of "truthful" or "deceptive" (auditors were asked to render only dichotomous decisions, that is, not to use "inconclusive" judgments as is sometimes the case in research of this nature). Following this latter decision, each auditor indicated the degree of confidence in the final decision. This was indicated on a 10-point scale, ranging from "1" no confidence to "10" almost certain. Statistical analyses were carried out using as dependent variables the auditors' truth/deception decisions and the confidence scores.

The sample of audio files used in this study was drawn from digital audio recordings of the pre-test interview segment of polygraph examinations. These interviews were collected by a police agency for the purpose of evaluating one of the commercially marketed voice stress analysis devices. Two persons employed by this police agency underwent the standard "Level 1" 40-hour training program offered by promoters of the voice stress analysis device. Each of these persons carried out blind analysis of the audio files using the voice stress analysis device. The voice stress analysis device results were used to judge if the interviewee was "truthful," "deceptive," or "inconclusive." This latter result indicated that the voice stress analysis device was unable to render a definitive outcome.

The voice stress analysis device decisions and the judgments rendered by the two auditors of the interviews were compared to two different ground truth criteria. The first criterion was, as is common in such research, a confession which implicated a "guilty" person and, in some instances, exonerated an "innocent" person in the same case. The second criterion was the result (that is, the decision yielded by analysis of the polygraphic data) produced by one of two commercially available computerized scoring systems (algorithms). The result of these algorithms, of course, provided a criterion that was free of the influence of examiners' assessment of the polygraphic data.

The results to be reported include the accuracy of the auditors' and the voice stress analysis device decisions. Also, the relationship of the auditors' decisions to their confidence and to characteristics of the suspects and the cases will be considered. Finally, the discussion will highlight what these findings suggest about research reports which are said to support the effectiveness of voice stress analysis as a means of "lie detection."

**Voice Stress, Deception, Police Interviews**

## D37 Measuring Typicality in Speech Features in American English Dialects: Towards Likelihood Ratios in Speaker Recognition Casework

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After attending this presentation, attendees will be informed about work in speaker recognition.

This presentation will impact the forensic community by describing new methods being developed to measure the typicality of certain features in American-English dialects.

This presentation will focus on new methods that are being developed to measure the typicality of certain features in American English dialects. The presentation will demonstrate the ongoing development in speaker recognition analysis. These methods have been enhanced by a growing knowledge of what is typical for various dialects in American English. The goal is to eventually build a large annotated corpus sufficient for establishing dialect norms for a variety of linguistic phenomena. It is hoped that such a corpus will assist forensic phoneticians and sociolinguists to quantify variation between and within American English dialects. It is also believe these methods will improve technologies for automatic dialect identification and automatic speaker recognition.

Claims about speaker recognition vary across multiple methods. In the field of forensic phonetics, applied phoneticians routinely identify speakers from phonetic characteristics that are hypothesized to be speaker specific.

Quantifiable norms of language- and dialect-dependent features are necessary for forensic examiners to assess if a given phonological or phonetic feature is speaker specific or commonly found in that speaker's dialect. To obtain these norms, detailed annotation of large sets of

speech data must take place. This research utilizes rapid, semiautomatic annotation techniques of detailed phonological and morphological phenomena for large-scale speech corpora. Resulting annotations and corpora will support both large-scale linguistic dialect analysis and automatic dialect identification.

The techniques used in the detailed annotation of large-scale speech corpora will be described in detail. "Regions-of-interest" (ROIs) were used, where an annotator is asked to make a judgment of whether or not a certain transformation of a given feature occurred in orthographically transcribed data. Transformations from General American English to a specific dialect are based on rules of occurrence in the specific dialect. These transformations are currently phonetic only and are among the most commonly occurring in spoken dialects of American English. Morphological rules will be applied at a later date. Phonetic rules were developed by a team of sociolinguists and sociophoneticians. The use of ROIs, together with an annotation tool, allows a large amount of data to be processed in a shorter amount of time. The output of these judgments is a likelihood ratio between speech samples. With enough judgments from the speech corpora a measure of typicality can be used, allowing for likelihood ratios between speech samples and compared against a given population.

The ROI method has been carried into speaker recognition casework for testing purposes. Each case starts with a detailed orthographic transcription. The transcription and original audio are processed to create a file where the audio is aligned with a transcript that contains a word layer and a phone layer. Once the transcription is checked for errors the word and phone layers are fed into a tool to generate ROIs based on the above-described rule-development process. The ROIs are then judged by an expert examiner in a different tool. It was found that the process must be carried out by expert examiners with backgrounds in linguistics and phonetics since judgments must be informed and interpretation of output is highly technical in nature. The presentation will lay out each step in the analysis procedure from raw audio to final conclusion.

**Speaker Recognition, Likelihood Ratios, Linguistics/Phonetics**

## D38 How to Train a Facial Recognition Examiner

*Nicole A. Spaun, PhD*, Federal Bureau of Investigation, Forensic Audio, Video and Image Analysis Unit, Building 27958A, Pod E, Quantico, VA 22135*

After attending this presentation, attendees will have an understanding of biometric facial recognition and the disciplines that a forensic examiner should know to perform facial comparisons.

This presentation will impact the forensic community by giving a brief overview of biometrics and the state of the art in facial recognition. In addition, the work will outline the important topics used in training forensic examiners to perform facial comparison examinations.

The recent increased availability of automated facial recognition systems appears, on the surface, to be a boon to law enforcement. However, the accuracy of these systems varies greatly, particularly when dealing with real-world surveillance images. Therefore law enforcement must be careful that facial recognition systems do not lull the community into false security. No facial recognition system, for private or government use, is ready to be run 'lights out' and provide accuracy rates acceptable for the judicial system. Thus the output of any facial recognition system must be verified by a human examiner. This can be accomplished in the field, e.g., by a police officer comparing the output of an automated system searching a database of arrest photos to the subject the officer has just pulled over. This verification can also be accomplished in a forensic lab, e.g., by an image analyst performing the one-to-one comparison of the output of a facial recognition system to the subject observed in a surveillance video. These practices are akin to using the IAFIS system, where latent print matches from the system are generally checked by forensic examiners. However, unlike in the fingerprint community where there were already numerous fingerprint examiners working, such that automation decreased the number of examiners needed, the facial identification community is exceptionally small, where most forensic labs do not have image analysts trained to perform these comparisons. Therefore, automated facial recognition will actually increase the number of facial identification examiners needed. These forensic examiners will need sufficient training to compare human faces and have the results accepted in a court of law.

Facial comparison examinations have been performed at the Federal Bureau of Investigation for at least 40 years; FBI examiners have testified in court to such comparisons nearly as long. The training that goes into forensic facial comparison examinations is robust. The training curriculum used by the FBI includes the following key topics: the anatomy of the human head, the nature of aging and alteration, the principles of imaging science, the scientific principles of comparison, and the methods of comparison. Each of these topics can be further subdivided into critical areas. For example, anatomy of the head includes learning the bones of the skull, muscles of the head, dermatology of the head, and properties of the ear. Image science is a necessary component that includes areas such as understanding image processing, compression, and resolution, and also awareness of perspective, illumination, and optics. The combination of these topics allows the examiner to have a broad knowledge base, to assist in performing the comparisons. The curriculum also includes the history and legal basis for such comparisons, to assist the examiner in testifying in court.

This work will present a brief overview of biometrics and the state of the art in facial recognition. In addition, the work will outline the important topics used in training forensic examiners to perform facial comparison examinations.

**Biometrics, Image Analysis, Facial Comparison**

## D39 Forensic Logistic Laboratory Process of the Mass Fatality Utilizing the Supply-Chain Operations Reference (SCOR) Model

*Robert D. Bytheway, BS,* Satellite Logistics Group, 12621 Featherwood Drive, Houston, TX 77034; and Joan A. Bytheway, PhD, Sam Houston State University, College of Criminal Justice, 1003 Bowers Boulevard, Huntsville, TX 77340*

After attending this presentation, attendees will understand the use of a logistical process based off of the Supply Chain Reference Model that can be used in a forensic laboratory of any mass grave or mass fatality.

This presentation will impact the forensic community by showing how a logistical process can be implemented into a forensic laboratory processing a mass grave or mass fatality. Attendees will clearly understand the logistical process that includes physical evidence and documentation flow, efficiency allocation of resources, leveraging of participant's qualifications, utilizing the process to maximize efficiency, and measuring process flow.

Both mass fatalities and mass graves require a very organized logistical method to efficiently and effectively process these types of events. The literature on mass graves extensively covers archaeological methods for proper field and excavation procedures utilized in mass fatalities/graves. However, little is found in the literature on operating procedures at a mass grave or mass disaster within the forensic laboratory. To produce accurate information with little or no compromise of evidence, and to combine the laboratory data to the data collected from the field, it is critical that there is a detailed and specific

laboratory process in place and that the laboratory be managed by an individual or individuals that are experienced in logistical processing.

In this presentation, the intent is to introduce the concept of utilizing the Supply Chain Operations Reference (SCOR) model in a forensic *laboratory* process of any mass fatality/grave and to take components within the supply chain model, define and build them into the development of a forensic logistical process. The SCOR model describes logistical principals that can be implemented without compromising accuracy, efficiency and chain of custody requirements. The presentation will focus on the specific steps in the SCOR model that correspond to the forensic laboratory process. This will include physical evidence and documentation flow, efficiency allocation of resources, leveraging of participants qualifications, utilizing the process to maximize efficiency, and measuring process flow.

Using the model in a similar manner as used in business applications, this model will provide a high level understanding of evidence flow through a forensic laboratory. This high level view provides the flexibility for varying applications, but provides a rigid outline for the understanding of sequential and dependent events. In addition, the distinction between the flow of evidence and flow of documentation will help to understand the importance of implementing this model in a mass fatality/grave scenario.

In a forensic scenario, there is a higher sense of purpose within the processing which can stimulate the efforts and willingness of participants. Participants are willing to perform many duties for the greater good of the mission, regardless of what the duty might be or how it affects the complete process. This however, can be detrimental to the successful completion of the project. Participant's willingness to perform many of these tasks cannot alone, carry the successful processing and completion of the mass fatality/grave.

If all mass fatalities have a similar structured logistical process, universally accepted (such as the SCOR model) it will provide predictable and comparable information that can be analyzed and used to establish a benchmark standard. Government or non-government organizations that fund mass grave/mass fatality endeavors will be able to measure efficiency, to determine if they are within budget and within the time frame for the project.

**Supply Chain Operations Reference Model, Mass Graves/Fatalities, Logistical Process**

## D40   SOBER: A Virtual Collaboratorium for Synchronous Online Biomedical Education and Research

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The goal of this presentation is to demonstrate new technologies that have successfully enabled interactive real-time collaboration and teaching between researchers, experts, trainees, and forensic agencies in a virtual environment, as well as provided a forum for forensic experts from around the world to meet and collaborate without requiring close physical proximity.

This presentation will impact the forensic community by gaining scientific knowledge of new technologies and methods, ultimately improving access to resources and opportunities for research normally impeded by geographic location. Advantages include the ability for law enforcement to choose experts based on suitability rather than propinquity, and for students to access a wide range of teaching collections and experience housed in multiple institutions.

Current telecommunication technologies have provided a means for forensic researchers and professionals to conduct research and provide educational training beyond actual physical contact.[1] Collaborators at the Universities of South Florida and Liverpool John Moores University have instituted a pilot program, the SOBER (Synchronous Online Biomedical Education and Research) Collaboratorium, that has been enhancing teaching and learning through virtual classroom and laboratory environments. This technology has also been used to conduct research collaborations through synchronous sessions with international experts via the internet.

Remote technologies have been explored in the medical field for clinical, research and military purposes. Over the course of a year, an international team of researchers were able to collaborate on forensic research and casework, while testing different methods of online remote telecommunication tools. A software package, that is an online, educational and collaborative program that allows for real time communication for multiple users ranging from simple one on one interactions to mass communications consisting of groups of 200 or more. One of the strengths of the software is that it is not bound to any specific computer platform, level or internet connection speed.[1] Synchronous sessions have also provided opportunities for remote use of high end sophisticated imaging software packages utilized by the collaboratorium for three-dimensional modeling, visualization, and analysis.

In the virtual classroom, the researchers have been able to create virtual anatomy and anthropology laboratories using reliable virtual models created by the lab[2] and then share that content between universities. Lectures and practicals can be conducted simultaneously in both countries and teaching expertise shared using the academic software. This online academic software can be used alongside any online training courses to create a multimedia, interactive environment for the trainees. Students have live access to the instructors no matter where they are located.

In an era of budgetary concerns, it is becoming less feasible for local forensic agencies to obtain access to leading experts from around the country and the world. Virtual collaboration tools will provide the ability for these agencies or institutes to collaborate without having to bring the expert physically to the agency. In this study, data was securely transferred and analyzed on proprietary software by the researchers via remote computer access. Online voice and video conferencing served as brainstorming and feedback sessions for the virtual laboratory.

As the world gets smaller via new communication technologies, researchers will have new opportunities to expand their networking capabilities beyond their local agency, institute, or university. Access and the ability to share these high end resources will prove to be invaluable tools in the progression of scientific research, training and collaborations.

**References:**

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**Computers, Remote Research, Technology**

## D41 A Review of Forensic Science Programs in the United States

*Kristen L. Tregar, MS\*, and Gloria Proni, PhD, John Jay College of Criminal Justice, 445 West 59th Street, Science Department, New York, NY 10019*

After attending this presentation, attendees will learn information regarding the characteristics of the bachelor and master forensic science programs.

This presentation will impact the forensic community by providing knowledge of the forensic science education in the United States.

Over the last 30 years, the number of institutions offering forensic science higher education programs has increased from 21 to 120. However, despite an increase in student interest and program availability, there has been a consistent reluctance to hire individuals with degrees in forensic science. This is due in part to a lack of information available about these programs, in terms of course offerings, equipment available to students, degree or certificate requirements, and other important aspects of the programs. Additionally, while accreditation by the Forensic Science Education Programs Accreditation Commission (FEPAC) ensures adherence to certain standards, it is not required. As a result, it is possible for the curriculum of forensic science higher education programs to vary considerably.

To obtain an understanding of the variance observed in forensic science higher education programs and their course offerings and requirements, the existing academic Forensic Science programs in the United States were invited to participate in an electronic survey. The survey requested information regarding the number of courses, subject inclusion, pre-requisites, degree requirements, available instrumental and academic resources, and experience, degree level, and participation of faculty.

It was found that, of the responding institutions, relatively few of them are FEPAC accredited, although most intend to apply or have applied for accreditation. It was also observed that, in general, the responding programs vary considerably in terms of their size and subject coverage.

Following this presentation, attendees involved in forensic science higher education programs will have a greater understanding of the offerings of other institutions, and may subsequently choose to adapt their curriculum such that greater standardization of degree requirements would be possible. This would enable laboratory directors and supervisors to better understand the qualifications of students graduating with a degree in forensic science.

**Forensic Science, Education, Standardization**

## D42 Teaching and Assessing Ethics and Law Within Medical Education: Implications in the Arab World

*Amal A. Mashali, MD\*, Mu'tah University, Faculty of Medicine, Alkarak, JORDAN*

After attending this presentation, attendees will gain knowledge on how the quality of medical education is ultimately judged by the ability of its graduates to perform at a high level. Graduates must be able to care for individual patients. They must be able to work effectively, competently, and safely in a diversity of cultural environments. Thereby, graduates must possess a sufficient educational base to respond to evolving and changing health needs throughout their careers. For this reason it was determined that ethics and law should be introduced to students. With the knowledge of ethics and law the students could better understand their own professional and legal responsibilities when working with patients.

Forensic medicine is the medical specialty that links medicine with the law. In today's increasingly litigious society, newly qualified doctors should not start practicing before having received a basic grounding in medico-legal matters. The application of ethics and law to medicine is now an emerging academic discipline with intrinsic and rigorous standards.

The general objective of the course is improving medical care and medical education by building greater awareness and understanding of the moral, ethical and social dimensions of medicine with reference to the law governing some medical conditions. The contents and strategies were in accordance to the global standards of medical education.

The tutoring methods used were lectures, using audio-visual aids as PowerPoint presentations, small group discussion in addition to problem solving and case studies as a form of problem-based learning in realistic clinical cases. The cases emphasize ethics, but also include human behavior, basic science and clinical medicine.

The evaluation strategies were formative assessment, in the form of quizzes to assess intellectual skills, summative assessment in the form of MCQs to assess intellectual skills, and problem solving to assess professional skills.

The contents of the course were eight Instructional Units:
I. Historical review of the evolution of medical ethics
II. Basic principles of medical law, medical ethics and health care ethics
III. Ethical responsibilities of physicians
IV. Issues related to patient autonomy as Confidentiality, Consent for treatment, Brain death (definition, criteria), and Ethics of organ transplantation from living and dead in addition to Euthanasia (active, passive, assisted suicide, withholding resuscitation)
V. Ethics of Reproductive Medicine and the legal condition of the fetus
VI. Medical documentation (Medical Records)
VII. Legal responsibilities of physicians and Malpractice (definitions, elements and basis of evaluation)
VIII. Ethics of medical research, related to the research itself or to the subject of research, whether human, animal, tissue, genetic material or fetal tissue

**Medical Ethics, Medical Education, Global Standards**

## D43 The Symbolism in Mafia Homicides: The "Violation" of Mafia's Honor Code

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The goal of this presentation is to show how a mafia homicide, in which the brutality of the crime, is associated with a ritualistic symbol. Symbols and signs are used in many life and societal aspects. They are very important codes, especially in some criminal organizations where they become an advertisement and a warning for all and especially for the experts or "adepts."

This presentation will impact the forensic community by illustrating a case which resulted in death that occurred in Palermo during the eighties. The victim, a male and an "uomo d'onore", was well known in criminal circles. The victim was like a "u cantante" (singer) because his criminal activities were akin to a singer of melodic Neapolitan songs. The victim was found dead, in a little place in

Palermo, with his genitals in his mouth. The autopsy showed that the victim was initially beaten than strangled and after he expired he was emasculated.

"Cosa Nostra", as the Mafia is defined by "her adepts", is a criminal organization which was born in Southern Italy, according to some authors, after the Italian unification in 1861. According to historical sources, in the South of Italy (Sicilia, Campania, Calabria), some criminal organizations were already present, originating from the Roman or the Arabian dominations. The progressive expansion of "*Cosa Nostra"* was increased by the application of new Italian laws. These laws, which forced those in agriculture to divide the fields between farmers, created a progressive malcontent amongst the farmers.

The farmers had to pay a tribute (the "*gabella"*) to the few owners of the fields for the privilege of working the fields. The collection of this tax was made by the *"campieri",* unlike what happened in the other Italian regions. The progressive control of the economy and increased power of the privileged class was established by the relationships they formed with the Statesmen, who resided in their territory.

This first phase was followed by a crisis, during the Nazi-fascist domination between 1920 and 1940. After the liberation of Italy from Nazi-fascist control, "*Cosa Nostra"* reached its definitive organizational structure in the early seventies thanks to different economic operations (such as control of the drug market, tobacco smuggling, control of business contracts). In order to avoid conflicts of interest, in this phase, the Mafia took on a pyramidal business structure. At the base of this "Cupola" there were men well known as "*uomini d'onore"* (honor's men, the old "*campieri"*): they are quite the soldiers, totally obedient to "*Capodecina"* a sort of a peripheral chief; all chiefs and soldiers composed the "*Famiglia"* (Family), that has some delegates, called "*Capimandament."* These delegates gather to decide strategies and actions. Recently, because of the Mafia's growth and the peripheral expansion, it has created the "*Commissione interprovinciale"*, in order to coordinate its many districts' actions.

After the rituals of initiation, each member has to comply with some specific rules that constitute the "Codice d'onore" (honor code). The honor code states that they must respect the rules of the organization, respect the others ("*uomini d'onore"*), and uphold the obligation of silence about the Organization. Finally their duty to women, especially for those who uphold their family and children are likened to the "man of the state", like policemen, judges, who have always been "untouchable".

This presentation illustrates a case of death which happened in eighties in Palermo, in which a man, an "uomo d'onore" known in the criminal society circles like "u cantante" (singer) for his activity like singer of melodic Neapolitan song, was found dead, in a little place in Palermo. The victim was found with his genitals in his mouth.

The investigations did not identify the killers and the investigators believed that the murder was due to a "sgarro" (bad action) due to a courtship of a woman who was from another "family", and thus was considered untouchable. The gesture of placing the genitals in the mouth was an expression of the will to make the facts known.

The goal is to show (or explain) the structure of Mafia's organization, its rites and the symbolism used in the Mafia's use of homicide. Other cases in which the symbolism was linked to the violation of a specific rule in the Mafia's honor code will be shown.

**Mafia's Honor Code, Emasculation, Signs and Symbolism**

## D44 Mafia Homicide During the 80's and Early 90's (1981-1985 – 1990-1992): The Unusual Use of War Weapons (Kalashnikov AK-47) – An Analysis of Murder Cases in Sicily

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The goal of this presentation is to show how some of the most important murders that were carried out by Cosa Nostra through limited use of the AK-47. Recent investigations sanctioned by the judiciary system have shown that Corleonesi once had a large arsenal stocked with automatic weapons, AK-47s and other weapons from countries of Eastern Europe. Now murders are no longer committed with this kind of weaponry in the Sicilian territory.

This presentation will impact the forensic science community by showing the characteristics of the machine gun, the ammunitions used, and the wounds found on dead corpses.

The 1980s and the early 1990's were an interesting period in the Mafia's history which saw the use of weapons never use before (Kalashnikov AK-47).

The use of war weapons was born from the demand of some Mafia families to prove to the Government, and to their adversaries, their "military power." In fact, before this period, Mafia's homicides were committed by the use of hunting weapons, "normal" handguns (cal. 38 special/357 magnum) or sometimes a machine gun stolen from the police. Nevertheless, the greatest economic power, obtained from drug trafficking, allowed the most powerful families the opportunity to purchase weapons from countries of Eastern Europe.

The term "Cosa Nostra" usually indicates a criminal organization, located in Sicily, that originated in the beginning of the nineteenth century, and became an international organization in the second half of the 1900s. To mark the change in the structure and methods of the "Cosa Nostra" there was a transition from cigarette smuggling to drug trafficking, which was very profitable. This caused a war inside the Mafia, between the old historic Mafia, composed primarily of some families from Palermo (affiliated to Bontade, to Badalamenti and Buscetta), and the Corleonesi (whose leading members were Luciano Liggio, Bernardo Provenzano, Salvatore Riina, and Leoluca Bagarella). Corleonesi was a really ferocious group, who demonstrated its power by making a series of well-executed murders. These murders were carried out on anyone who might constitute and obstacle, like judges, politicians, policemen and journalist.

In the execution of the classic Mafia crimes, the members would use .38 special caliber pistols or .357 Magnum and 12 caliber shotguns, which were loaded with buckshot (" lupara"). At the beginning of the 1980s automatic rifles made their appearance, in particular submachine guns with a caliber 7.62 x39, the model AK 47 and its derivatives (AKM 47). These rifles were used for the first time to murder the leaders of the Mafia's competition and in the commission of other crimes.

**Kalashnikov AK-47, Homicide, Mafia**

## D45 Denial in Scientific Inquiry and Its Impact on Forensic Science

*Mark Feulner, MS\*, Underwater Crime Scene Investigation-Florida State University, 4750 Collegiate Avenue, Panama City, FL 32405*

After attending this presentation, attendees will understand the means by which the impediments to scientific exploration and

advancement in forensic science can be categorized and examined.

This presentation will impact the forensic community by facilitating the advancement of forensics as a science by providing a philosophical tool for the evaluation of the controversies that surround the emergence of innovative perspectives, groundbreaking methods, or other new ideas and approaches.

Thomas Kuhn contributed greatly to the philosophy of science in describing the purpose of scientific endeavor, how science progresses, and the manner in which scientists respond to emerging theoretical perspectives. His explanation of paradigm shifts in science involved the accumulation of facts counter to the reigning theoretical perspective, the development of new theories to challenge the old, and the eventual adoption or incorporation of the new perspective. In explaining this process, Kuhn presented the problem of denial, but failed to elucidate. Denial exists at each stage of his model for scientific revolution, and the mechanisms by which it is employed must be determined in order to develop a useful understanding of the phenomenon.

Various forms of denial plague the scientific community in general, with its effects expressed on three levels. Denial hampers scientific inquiry by: restricting research and investigative activities either through individual bias, institutional norms, or professional standards; fostering irrational controversies and illogical criticisms to nontraditional work; and preventing or damaging the validation of various disciplines as worthy of consideration as scientific endeavors. These consequences of denial can be seen at all three levels in the field of forensic science. Therefore, the questions that must be asked are: What are the mechanisms of denial? Why do reputable scientists engage in denial? What is the impact to forensic science? In order to answer such questions, the activity of denial in science must be categorized so that its function may be deconstructed and examined.

The use of denial in the realm of science is analogous to the states of denial espoused by Stanley Cohen in his explanation of why good people fail to prevent or otherwise cause bad things to happen. His various forms of denial can be categorized in a matrix by focusing on two issues, the level of knowledge and the level of malice involved in the action or inaction in question. Identification of the amount of malice and knowledge involved in any given act of denial identified the specific category into which it may be placed. Such a matrix serves as a model for examining denial in scientific inquiry.

Scientists have various reasons for their denial of the reality apparent before them. The impact to forensic science is seen in the approaches taken to casework, to education, and to training. Further damage is done in opening the field to criticism from without by other criminal justice practitioners and academics. This paper presents a matrix for the categorization of scientific denial, which may be readily extended to forensic science. In doing so, it also provides a means by which the constraints to scientific endeavors may be countered, and thereby enrich the forensic community with greater latitude while bolstering the community itself. At the heart of this discussion is the argument that philosophy should not only be a part of forensic science, it is a critical component.

**Philosophy of Science, Bias, Research Reliability**

# E1 Whores of the Court – Revisited

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After attending this presentation, attendees will understand the criticisms of behavioral science expert testimony and evidence, the application of *Daubert* and its progeny to behavioral and other "soft" sciences, the controversies surrounding behavioral evidence, how behavioral experts work with the courts, the role of behavior in American jurisprudence, and emerging trends in scientific evidence and its admissibility as it relates in particular to human behavior.

In the United States alone, expert behavioral science testimony is presented in some 2.5 million civil and criminal trials each year. This presentation will impact the forensic community by demonstrating how by this measure, behavioral science is the most frequently applied forensic science; and it is also the most criticized and controversial. In 1997, Professor Margaret A. Hagen leveled her criticisms of behavioral testimony in her book **Whores of the Court: The Fraud of Psychiatric Testimony and the Rape of American Justice**. In this session, Professor Hagen with appear to reprise and sharpen her earlier criticisms.

Since Hagen's book was published, the *Daubert* holding and its progeny have changed the rules related to the admissibility of scientific evidence, and made judges gatekeepers with the power to bar experts proffering testimony and evidence that fails to meet proper scientific standards. While these changes have had an impact on the testimony presented to jurors, behavioral science testimony has not significantly abated. Prof. Jane Campbell Moriarty, the author of several books on behavioral evidence, will follow Hagen's presentation to provide a scholarly overview of the evolution of the U.S. Judiciary's acceptance and application of such testimony, as well as its varied consequences.

Psychiatrist William Bernet will continue with a presentation on the actual role and experiences of behavioral experts in practice and before the courts. Dr. Bernet will draw upon his extensive history formulating and presenting psychiatric testimony in civil and criminal litigation.

Carl N. Edwards, an attorney and forensic psychologist, will conclude the formal presentations with an overview of behavior and the law, tracing its history to before the founding of America, and discussing why the U.S. is unique in the world in its use of behavioral experts. The significance of human behavioral concepts in American jurisprudence, the legal distinctions between behavioral and other sciences, and the reasons why finders of fact turn to behavior experts will all be considered and placed in context to provide predictions as to where the courts will move in this controversial field during the years ahead.

These four presentations will be followed by a panel discussion in which forensic psychologist Daniel A. Martell, PhD and psychiatrist Robert Weinstock, MD will join the presenters.

Andre A. Moenssens, JD, LLM, Douglas Stripp, Professor of Law Emeritus, will moderate both the scientific presentations and the panel.

This session will not only provide a forum for the first serious debate of one of the most hotly contested issues in the forensic sciences, but it will provide an overview of the scholarly multidisciplinary research in the field, and examine conceptual and operational concerns fundamental to forensic sciences and their future as a whole.

**Admissibility, Behavior,** *Daubert*

# E2 The CSI Effect in the Australian and Canadian Criminal Justice Systems

*Judith Fordham, LLB\*, Murdoch University, School of Biological Sciences & Biotechnology, Murdoch, 6150, AUSTRALIA; and Janne A. Holmgren, PhD\*, Mount Royal College, Department of Criminal Justice Studies, 4825 Mount Royal Gate South West, Calgary, T3E 6K6, CANADA*

After attending this presentation, attendees will gain insight into the particular issues Australian and Canadian jurors face when dealing with complex scientific evidence. The issues faced by jurors are mainly related to what they watch on crime-related television shows. In particular, this session will inform attendees about the influence of television on the understanding of forensic evidence.

This presentation will impact the forensic community by providing information about the target audience which can assist in framing reports and courtroom presentations (giving evidence). The information provided in this session will assist the forensic community and the ends of justice generally by providing an insight into the knowledge base of jurors, permitting an adaptation of focusing of "teaching" style used by lawyers and experts in court.

The preliminary findings suggest that potential jurors are educated, but not always correctly, about forensic evidence from watching television shows. The concerns raised are amenable to solution, provided forensic scientists and lawyers are aware of the misconceptions which arise. Benefits of exposure to such shows were also apparent especially in interviews with Australian jurors who showed a willingness to engage with unfamiliar technical or scientific evidence, and a healthy scepticism in relation to the more extravagant claims of such programs. Differences in both information and misinformation were apparent between those who watched such shows and those who did not.

The purpose of this research project was to develop insight into the factors that influence jury interpretations, perceptions and understanding of forensic evidence within the Canadian and Australian criminal justice systems. Television shows such as CBS's CSI and its spinoffs CSI: Miami; CSI: Las Vegas, and CSI: New York has sparked the imagination of thousands of students who want to become forensic scientists. The shows' fictional portrayal of crime scene investigations has prompted real demands for DNA and other scientific evidence from prosecutors and defense lawyers in the courtroom. It's what lawyers and judges refer to as the "CSI effect." This phenomenon was studied using a triangulated data collection methodology involving the following: the collection of television guides and reports, which included an examination of the content of the crime-related shows; the distribution of 605 surveys to College students who would be considered jury eligible; and the qualitative findings from interviews with real jurors. This data provides the background and preliminary findings of how crime-related television shows might contribute to whether or not the so-called CSI effect presides in the minds of Canadian and Australian jurors.

**CSI Effect, Evidence, Juries**

## E3    *Daubert* Challenges to Fingerprint Science: Legal and Scientific Update

*Thomas L. Martin, BS\*, Crime Scene Forensics, LLC, PO Box 515, Red Hook, NY 12571; and Stephen P. Hogan, JD\*, New York State Police, Building 22, State Campus, 1220 Washington Avenue, Albany, NY 12226*

After attending this presentation, attendees will understand the issues being raised worldwide regarding the reliability of fingerprint science. Recent court decisions have generated questions regarding the consideration of fingerprint identification as a reliable science. *Daubert* hearings are now challenging a science that has been considered sound for over one hundred years.

This presentation will impact the forensic community by explaining the basis behind the use of fingerprint identification as a reliable science and addressing the issues raised in recent *Daubert* challenges.

The science of fingerprint identification has been widely accepted by the scientific community and courts of jurisdiction since 1903, when fingerprints replaced the failed Bertillon system as the method for identifying individuals. The use of fingerprints as a means of identification dates back hundreds of years, but it was Sir Francis Galton in 1892 who outlined the unique characteristics of friction ridge skin, and their application to positive identification. Since that time, fingerprints have been used to identify individuals arrested and imprisoned, to convict the guilty, to exonerate the innocent, and to positively identify human remains. Governments around the world use fingerprints to identify individuals for purposes such as: employment, military service, adoption, and licensing. Fingerprints have long been accepted by those who work in the criminal justice system: police personnel, attorneys, and judges alike.

The scientific basis for the use of fingerprints as a positive means of identification is that they are permanent and individually unique. That is, the friction ridge skin begins to form on round, volar pads on the fingers, palms, and soles of the feet, during the 6th week of gestation. The friction ridge skin is formed as a result of arbitrary stresses and pressures against the volar pads during fetal development. The differential in these stresses and pressures are what make the ridge formation unique. Even in the case of identical twins, all twenty fingerprints will have unique ridge arrangements. Barring accidental or surgical removal or permanent scarring or deformation of the fingers, the ridge arrangement of an individual will remain permanent from gestation to decomposition after death.

With the introduction of the *Daubert* standard in 1993, a realization occurred to the legal community fingerprint science had never been subjected to the *Daubert* standard. That is, the science was just accepted, but the specifics substantiating the science as reliable had never been put on record. Fingerprint experts repeatedly submitted the same innocuous explanation in their expert testimony – "it's reliable because it's fingerprint science." The basis for the science has never been detailed or articulated for the court system, and judges, with a new gate keeping role, were obligated to have that foundation of fingerprint reliability detailed for the record.

In the recent case of NH vs. Langhill, the trial court judge ruled a particular fingerprint identification as unreliable, as the verification phase of the scientific method employed by the investigating agency, was biased. That is, the verifying fingerprint expert had been advised by the case examiner that an identification had been made to a particular finger; prior to the verification procedure being completed. This ruling, which has since been reversed by a higher court, set off the idea of using blind verification in the fingerprint identification methodology.

The reliability of fingerprints as a science is quite sound; it is the basis for the reliability that needs to be more clearly articulated. The lack of a detailed, scientific methodology for fingerprint identification has created a wave of challenges to an existing staple in individualization. Recent legal challenges have raised issues that will be addressed in this presentation. Further, the scientific basis behind using fingerprints as a means of identification will be explained, as will the position facing attorneys, judges, and police investigators.

**Daubert, Fingerprints, Identification**

## E4    Science Approach to Applications of Bloodstain Pattern Evidence With Special Regard to *Daubert* Qualification

*Anita K.Y. Wonder, MA\*, Wonder Institute, PO Box 1051, Carmichael, CA 95609-1051*

After attending this presentation, attendees will learn how bloodstain pattern evidence may be used with suggestions for *Daubert* error rate qualification, notes for trial preparation, and ideas for effective use of experts in this very probative discipline.

This presentation will impact the forensic community by providing knowledge regarding the nature and application of bloodstain pattern evidence within the adjudication process.

At the 2008 Washington, D.C. meeting of the AAFS, a multi-disciplinary panel discussion was presented by a group of experts who discussed the qualifications for pattern match types of evidence within *Daubert* requirements. Although the primary focus of that session was fingerprints, at least one panelist suggested that bloodstain pattern evidence was also a subject of concern under the topic. Such association leads to a need for clarification in the legal handling of this form of physical evidence.

The first consideration emerges from the fact that bloodstain patterns are not similar to fingerprints. Although the evidence is analyzed as "patterns," the dynamics in which blood is distributed is substantially different from those acts which form fingerprints and tool mark evidence. From a true science viewpoint bloodstain pattern evidence is not a pattern match form of evidence, nor is it limited to "blood spatters." The fact that it is often applied and mistakenly accepted as such has lead to considerable misunderstanding in legal application.

The need for special handling in a *Daubert* jurisdiction; however, does require discussion. Establishing an error rate is one area in which bloodstain patterns require attention to details in a manner similar to but also substantially different from pattern match formats. As with any expert testimony the concern for disclosure of errors is offset by reluctance to point out possible weaknesses in testimony to opposite counsel. A suggestion was made at the Washington, DC meeting to supply a list of sources of error for the court approval, while also supplying representative counsel with explanations how each possible error was recognized during the analysis being qualified. This paper will attempt to illustrate how possible errors may be acknowledged while not discrediting a competent analysis.

Further benefit to the applications of bloodstain pattern evidence in violent crime adjudication is the rationale for using expert witnesses. Too often the defense assumes that there is only one possible approach, and the prosecution will cover it. Benefits to the defense exist in convincing a client to accept a negotiated plea, gaining reduced charges, or proving the client not guilty as charged. It is often possible for a defense expert to aid counsel in examining the state's experts even if testimony from an additional expert is not required.

This very probative form of evidence benefits the judicial community and should be understood separately from "trace evidence" or "police work." This paper will focus on the specific objective applications available with bloodstain pattern evidence rather than the simple approach to "bloodspatter analysis."

**Bloodstain Pattern Evidence, Bloodstain Pattern Expertise, Trial Preparation**

## E5 The Role of the Forensic Expert in Criminal Cases Pursuant to the Sixth Amendment of the U.S. Constitution

*David M. Benjamin, PhD\*, 77 Florence Street, Suite 107, North, Chestnut Hill, MA 02467-1918*

After attending this presentation, attendees will learn the Confrontation Clause of the *Sixth Amendment*. Attendees will examine the role of the criminal defense expert under the *Sixth Amendment*, and identify the requirements of the Brady Doctrine.

This presentation will impact the forensic community by demonstrating the criminal defense expert's pre-trial role in "confronting" the scientific evidence against a defendant, as described in the *Sixth* and *Fourteenth Amendments* of the U.S. Constitution.

The confrontation clause of the 6th Amendment of the U.S. Constitution guarantees every criminal defendant the right "to be confronted with the witnesses against him" at trial. However, the defendant's right to a critical review of the evidence to be offered against him/her at trial begins during preparation for court, long before the trial. This is consistent with the practice of initially retaining experts as consultants and not as "testifiers." Accordingly, when retained by the defense, the expert's first role is to study the evidence and figure out what happened, and then to explain to the defense attorney how inculpatory the evidence is and how strong a case the government will be able to mount against the defendant at trial. While the right to retain a forensic expert may derive from the due process provision of the 14th Amendment, the pre-trial review of evidence which will be used against the defendant in court is an extension of the 6th Amendment of the U.S. Constitution. This right of confrontation includes reviewing, examining, and assessing the actual physical evidence or results of laboratory testing that will be the subject of expert testimony against the defendant at trial. In the field of forensic toxicology, such evidence frequently includes the results of breath ethanol tests, blood and urine drug tests, and laboratory reports describing the weight of contraband such as heroin or cocaine found in the possession of the defendant. In some cases, after a review of the evidence, the expert may form opinions that are favorable to the defendant's case, and in other instances, the expert may not have anything helpful to say. After completing a review of the evidence, only then is the expert in a position to decide if he/she will testify at trial.

In criminal cases, most defense attorneys agree that keeping potentially inculpatory evidence out of trial is a far better tactic than trying to mitigate it once it has come in. Therefore, it is incumbent on the criminal defense attorney to work closely with the forensic expert in order to prepare and present pre-trial motions to suppress unreliable evidence, or file a "*Daubert* or *Frye* Motion" to limit or suppress potentially inculpatory testimony that "misleads the jury or is unduly prejudicial" as described in FRE 403. To propound such a motion, the defense attorney needs the assistance of the forensic expert, since only the competent forensic expert is in a position to recognize "junk science" and point out its lack of reliability to the attorney.

Although it may be necessary for the defense expert to testify at a pre-trial hearing to point out the lack of reliability of certain evidence the government may want to present in its case in chief, that does not mean the defense expert will testify at trial. Testifying at the pre-trial hearing is necessary to establish the standard of care in the forensic community or the lack of reliability of the government's proffered evidence. In this capacity, the expert is concerned with helping the court recognize the lack of reliability of certain proffered evidence, test results, or incorrect opinions that the government wants to offer through its forensic expert(s). Such a review is also provided for under the Fundamental Fairness doctrine which resides within the 14th Amendment.

Recently, public attention was focused on the "Duke rape cases" where the prosecutor knew he was moving against innocent defendants and also withheld exculpatory evidence to that fact from the defense, despite a duty to comply with the Brady Doctrine. That prosecutor has lost his license to practice law in North Carolina, and will always be remembered as the part of the horse that went over the fence last! The Brady Doctrine derives from Brady v. Maryland, 373 U.S. 83, 83 s. Ct. 1194, 10 L. Ed. 2d 215 (1963), which creates a due process requirement that exculpatory evidence be disclosed to the defense without a request from the defense to do so. In a subsequent case, U.S. v. Bryant and U.S. v. Turner 142 U.S. App. D.C. 132; 439 F.2d 642 1971, the U.S. Appellate Court explained that the pre-trial disclosure of evidence gathered by the Government "would make the trial more a 'quest for truth' than a 'sporting event.'" (Id at 642). In addition to the search for truth, the Bryant/Turner court also recognized that the government's duty to disclose arises from the need to even the playing field at trial due to the "imbalance in investigative resources" between the government and the defendant because much of the relevant material "will be exclusively in the hands of the government." (Id at 648) This is because the government is supported by the resources of the state crime labs, the FBI, and the DEA, while the defendant has to rely on one or two forensic experts who frequently must be retained with court-approved funds from the government prosecuting the case.

Our founding fathers devised the 6th Amendment in order to protect the rights of defendants, rich and poor alike, and the importance of that relationship still obtains today.

*Sixth Amendment*, **Confrontation Clause, Brady Doctrine**

## E6 Challenging Fingerprint Evidence — Legally and Scientifically: Is the Baby Being Thrown Out With the Bathwater?

*Judith Fordham, LLB\*, Murdoch University, School of Biological Sciences & Biotechnology, Murdoch, 6150, AUSTRALIA; and Marna Mclendon, JD\*, Arizona Attorney General's Office, 1275 West Washington, Phoenix, AZ 85007*

After attending this presentation, attendees will understand the scientific basis for challenging fingerprint evidence and be aware of the current status of legal challenges at trial stage. Attendees will be able to integrate the scientific method with the *Daubert* approach.

This presentation will impact the forensic science community by providing participants with a deeper understanding of the basis of the scientific method adhered to by all scientific witnesses and most expert witnesses, and echoed in standards for admissibility, including *Daubert*. Attendees should be able to integrate scientific knowledge with their legal or expert practices. Attendees will be in a position to critically evaluate this evidence and be aware of, mount, or guard against challenges, dependent on the nature of their brief. They will also be in a position to have a justifiable view on the question of whether there is any place for fingerprint evidence in a court and if so what that place is.

An international perspective on this vexed issue will be presented.

The legal challenges will be explored with reference to the ACE-V methodology (Analysis, Comparison, Evaluation, and Verification) and the Frye-Reed test (Maryland)).

In particular, questions of: (1) subjectivity (observer bias), (2) lack of objective criteria, (3) error rate, and (4) lack of independent review will be explored.

How these challenges are responded to and the variety of trial judges' decisions will be discussed, as well as the suggestions that problems with ACE-V method do not go to admissibility but are appropriate for cross-examination and go to the weight to be given to evidence.

This session also presents the scientific basis for challenges to fingerprint evidence. The claim by fingerprint examiners that fingerprint examination using ACE-V is critically examined:

- bases individualizations upon sound scientific principles
- gives conclusions are objective; based on faithful execution of this methodology, not mere observations or intuition
- assures validity and reliability of the conclusions.
- assures consistency & repetition of the methodology and
- embraces *all the factors required by sound scientific practices.*

The justifications for the claim that no two fingerprints are the same is examined, and compare and contrast each step of the ACE-V method with the accepted scientific method approach. The psychological basis for the existence of observer bias and the impossibility of removing this from fingerprint comparisons carried out as they are and have been will be discussed and demonstrated to participants. The change in some jurisdictions to a global comparison of prints, as opposed to a points approach will be considered and evaluated. Does this assist in *Daubert*-proofing the comparison?

A sample cross-examination based on the scientific method will be used as illustration, and case illustrations will be provided, both from the United States and internationally.

The challenges currently facing fingerprint analysis are expected to be launched in other areas of evidence based on comparative analysis and therefore the discussion is helpful for a variety of disciplines.

This presentation is suitable for defense and prosecution attorneys, comparisons experts, and forensic educators.

**Fingerprints,** *Daubert*, **Science**

## E7 Familial DNA Searching: Why Isn't the United States Embracing It? Issues and Answers

*Rockne P. Harmon, JD\*, 2846 Lincoln Avenue, Alameda, CA 94501; Frederick R. Bieber, PhD, Harvard Medical School, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115; Mitch Morrissey, PhD\*, Second Judicial District, 201 West Colfax Avenue, Denver, CO 80202; Greggory S. LaBerge, MSc\*, Crime Laboratory Bureau, 1331 Cherokee Street, Room 648, Denver, CO 80204; Gary A. Sims, MPH\*, California Department of Justice, DNA Laboratory, 1001 West Cutting Boulevard, Suite 110, Richmond, CA 94804; George W. Clarke, JD, 220 West Broadway, San Diego, CA 92101; Robert N. Green, LLB, MD\*, PO Box 28089, London, N6H 5E1 UNITED KINGDOM; and Stephen B. Mercer, PhD\*, 11 North Washington Street, Suite 520, Rockville, MD 20850*

This presentation will discuss the legal, policy, and philosophical issues related to expanding the crime-solving capability of our DNA databases by adding familial DNA searching. It will also present and discuss methods being used in the United Kingdom and in California to solve more crimes. The presentations will demonstrate that there are no sound reasons for not adopting these techniques, legal, policy, or technical.

This presentation will impact the forensic community by connecting all components of the criminal justice system and will forge a greater understanding of the topic than currently exists. It will force the topic out in the open where it belongs, rather than as a secret topic within the closed forensic community where it has lingered for too long.

At a time when considerable effort is being made to expand individual states' DNA offender databases, there has been scant public discussion of increasing the efficiency of existing database searches by conducting familial DNA searching that produce investigative leads to help solve crimes. In fact, Maryland recently enacted a greatly expanded database law in which there was an agreement <u>not</u> to allow for familial DNA searching.

This presentation will consist of an expansive set of presentations from experts in various fields who have been leading the effort to add familial DNA searching to individual states' crime-solving capabilities.

The presentations will include a representative from the United Kingdom who will discuss their scientific, policy, and investigative approach to familial searching that has been successful in about 10-15% of the cases in which it has been employed.

There will be a presentation from an experienced defense attorney who has voiced legal and policy concerns about familial DNA searching constituting lifetime "genetic surveillance" with a strong element of racial discrimination.

A presentation will be made by noted researcher Dr. Fred Bieber. Fred has written and lectured extensively on the subject. Fred will discuss the likelihood that brothers are present in offender databases, and general statistical approaches that will help identify closely matching DNA profiles as being from sibling pairs or father/son combinations.

A presentation from the Denver District Attorney, Mitch Morrissey and the Denver Police Department Lab Director, Gregg Laberge is also planned. Their presentation will include an analysis of familial DNA searches done using a local database and the contribution the searches had on solving crimes. There may also be a discussion about familial DNA searching research being done on the state level in Colorado.

California is the first state to have formally developed and adopted a policy and practice for conducting familial DNA searches in an effort to solve crimes. Gary Sims, from the California Department of Justice heads that program. He will discuss the technical and statistical issues inherent in the approach and will explain how his agency will implement them and apply them to assist law enforcement in solving cold cases.

There will be a presentation by San Diego Superior Court Judge George "Woody" Clarke concerning his opinion about legal, technical, or policy objections raised during the workshop or elsewhere

Finally there will be a presentation by recently retired prosecutor Rockne Harmon summarizing the presentations and discussing why this subject in not exclusively a laboratory decision and that it must be discussed and decided among the greater law enforcement community.

**DNA, Investigative Lead, Database Search**

## E8 "Touch and Transfer" DNA Samples: Practical and Ethical Issues

*Valerie K. Fahrnow, BSN\*, Clinical Reference Laboratory, 8433 Quivira Road, Lenexa, KS 66215*

After attending this presentation, the attendees will understand the practical and ethical issues of "touch and transfer" DNA in the judicial system.

This presentation will impact the forensic community by providing a better understanding of the analytical process, misconceptions, and powerfully persuasive evidential impact of the utilization of "touch and transfer" DNA in criminal cases.

Due to the continuing advancements of forensic DNA technology, evidence samples previously considered unlikely sources of DNA are now considered relevant and material. Attorneys must be aware of the developments and emerging standards of touch and transfer DNA, also known as low copy number DNA (LCN) and contact DNA. The analytical process, misconceptions, and evidential impact of low copy DNA in criminal prosecutions must be understood and properly utilized. Previously undetectable evidence is being presented to facilitate the administration of equal justice. However, forensic DNA is not a panacea. Limitations of touch DNA need to be recognized and respected. Selected practical and ethical issues of touch DNA will be addressed.

Touch and transfer DNA samples are minuscule amounts of DNA (< 50 pg) from any cellular or biological material which comes into contact with another object or body. Transference may occur from

person to person, person to object, object to person, and object to object. Due to the minute sample size, current testing procedures cannot adequately identify the origin (e.g., saliva, skin cells, biological fluids, etc.) of the DNA samples. Samples are collected with the intent of obtaining a DNA profile, regardless of cellular origin. Therefore, an assumption is made that potential cellular material exists and DNA analysis is used to validate its invisible presence. Common sources of touch DNA samples include: saliva on skin, fingernail scrapings, aspermatic semen, vaginal cells from penal swabs, shell casings, fingerprints, skin cells on ligatures, abrasion sites, gun grips, and perspiration stains. The possibilities and sources are nearly infinite.

The scientific principles utilized in typing these samples have been accepted by the judicial system for years using commercially available kits. Newly developed kits can differentiate between male and female DNA to aid in the elucidation of mixtures. Other kits are designed to type very small or degraded DNA samples.

The novelty and potential use of this rapidly emerging technology must be tempered through addressing practical considerations. These recognized scientific and analytical limitations include, but are not limited to: discretion in the selection of a typing kit, recognition of potential stochastic effects, the infallibility of low-level mixtures, adherence to established detection thresholds and compliance with analytical standards. Data analysis and impartial statistical significance of the results cannot be neglected. Presentation by the proponent, of these material factors must be competently and completely presented, in good faith, as an integral part of the judicial process.

In addition, ethical standards regarding the weight of the evidence must also be elucidated. Critical considerations for determining the reason for sample collection, issues of primary, secondary, and tertiary transfer, and sample contamination must all be considered. These issues foster speculation regarding evidential viability. Presence of a DNA profile, does not answer the question of when or how it get there nor its ensuing implications. The perseverance of introducing phantom suspects due to speculative testimony must be substantively examined. The potential of wrongfully convicting an innocent bystander or exoneration of a guilty person are of primary concern. The totality of the evidence is integral to the case. The mere existence of a DNA profile is not indicative of innocence or guilt.

The recognition and impact of touch and transfer DNA evidence in the judicial system is commonly neglected and misunderstood by the courts. In scrutinizing evidentiary standards for minuscule amounts of DNA in criminal cases, the court in State v. Freeman, 2008 WL 142299, (Mo.App.S.D. Jan. 16, 2008 - No.28150) determined, "DNA is robust and easily transferred ... Its mere presence is not adequate for inferences of guilt." Accordingly, prosecutors must be aware of limitations and challenges regarding touch DNA to minimize its misuse as evidence.

The analytical process, misconceptions and powerfully persuasive evidential impact of touch DNA in criminal prosecutions must be understood and properly utilized. Limitations of low level DNA need to be recognized and respected. The importance of ethical and good faith application of this invisible evidence is paramount.

**Touch DNA, Ethics, Evidential Standards**

## E9  Forensics for the Defense

*O'Brian C. Smith, MD\*, Conscience and Science in Medicine, LLC, 9639 Rosemark Road, Atoka, TN 38004*

After attending this presentation, attendees will understand the diversity of cases benefiting from a deconstruction oriented approach when evaluating prosecution materials, realizing that medical opinions are often made quickly, and bad facts, superficial observations, or lack of differential diagnoses require an integrated investigation to discern the strengths for the defense bar.

This presentation will impact the forensic community by giving a better understanding of the value of integrated investigations and their ability to serve justice.

Jurists for the defense are often faced with what appears to be a steep and slippery slope when considering the evidence arrayed by the prosecution with its endless resources of money, facilities, and experts. There are times when experts for the defense may have significant impact on the outcome, both at trial and in the appellate phase. Time permitting, six cases involving blunt force, sharp force and gunshot homicides, child abuse, vehicular homicide and DUI will be presenter.

The focus is on the process of deconstructing the proffered case by assessing the strengths and weakness in the state's use of its forensic capabilities; the need to develop sound differential diagnoses and alternative explanations, and ensure the standards of care have been met. The outcomes range from dismissal to the death penalty.

**Forensics, Homicide, Deconstruction**

## E10  Reducing the Probability of Mistake, Misunderstanding, and Conflict Between Experts and Attorneys

*Harry L. Miles, JD\*, Green, Miles, Lipton, & Fitz-Gibbon LLP, 77 Pleasant Street, PO Box 210, Northampton, MA 01061-0210*

After attending this presentation, experts who wish to serve as consultants and witnesses should expect to understand some of the major sources of mistake, misunderstanding, and conflict between themselves and attorneys. Attorneys should expect to understand the procedural, linguistic, and economic differences between their views of a case and those of the expert that lead to mistake, misunderstanding, and conflict. All participants should acquire a better understanding of how to avoid or to resolve mistakes, misunderstandings, and conflict.

This presentation will impact the forensic community by reducing mistakes, misunderstandings, and conflicts between attorneys and experts.

Experts who contract to consult with attorneys or who testify at the request of an attorney acquire certain rights and undertake certain responsibilities. As a result, questions and conflict may arise about the expert's performance of his or her obligations and the attorney's obligation to compensate the expert for his efforts. For example, may an attorney who obtains answers to interrogatories or deposition testimony from an expert use that material in other cases without compensating the expert? For example, may the attorney "cap" the expert's preparation time or fee? Should an expert "reserve the right to change my opinion" should additional information come to light? What obligation does the expert have to advise the attorney about the need for additional information upon which to base an opinion? How should the expert respond to an attorney who wants the expert to offer opinions the evidence will not support?

Misunderstanding about the rate or manner of compensation causes much of the conflict between experts and attorneys. The engagement letter or contract should resolve those issues in advance to reduce the probability of conflict. For example, whether the client or the attorney will underwrite payment should be clear before the expert performs. Whether a retainer is refundable, non-refundable, or partially refundable should be clear before the expert undertakes to perform.

The scope of the expert's opinion should be clear as well. The expert should inform the attorney about any general qualifications or limitations to the expert's opinion or methodology before any contract is executed or payment exchanged. For example, if a court has refused to qualify an expert or if an appellate court has rejected the expert's testimony, then that information should be provided to the attorney before the expert comes on board.

**Expert Witnesses, Expert Compensation, Expert Opinion**

## E11 Forensic Linguistic Expert Testimony in the Authentication of Language Evidence

*Blake S. Howald, JD\*, Georgetown University, Department of Linguistics, 37th and O Streets, North West, Washington, DC 20057-1051*

After attending this presentation, attendees will understand the relationship between forensic linguistic expert testimony, focused on author or voice identification of language evidence, and the underlying evidentiary considerations of authentication (e.g., Federal Rules of Evidence, Article IX) in the United States legal system.

This presentation will impact the forensic science community by providing a practical legal-scientific framework for understanding the expectations and limitations of implementing the linguistic analysis of language evidence.

In particular, focus on three related aspects of evidentiary jurisprudence which have been considerations in the inconsistent application of linguistics in the legal system: (1) the deference of the judiciary to the layperson's ability to determine if a document or recording is authentic, i.e., agree with what a particular party at trial purports a particular document or recording to be (e.g., Federal Rule of Evidence 901(a)), (2) the judiciary's ability to evaluate science generally (and linguistics specifically) in the acceptance, exclusion, or limitation of scientific expert testimony under standards of admissibility (e.g., the "general acceptance" standard of *Frye v. United States*, 293 F. 1013 (DC Cir 1923) or the "scientific sufficiency" standard of *Daubert v. Merrell Dowell Pharmaceuticals, Inc.*, 509 U.S. 579 (1993)), and (3) in certain circumstances, the judiciary's acceptance of non-scientist professionals who base their testimony on experience or expertise in lieu of scientific training.

Evaluation of the role of linguistic science from this evidentiary and procedural point of view, at all levels of the legal system (investigation, pre-trial and trial), reveals that often key participants in the legal system (law enforcement, judges and lay jurors) possess common misconceptions about language and, consequently, lack adequate knowledge to accurately evaluate the sufficiency of linguistic science. This observation is despite the existence of linguistic research in author and voice identification which is empirically based and provides validated results that are consistent with the contemplation of scientific sufficiency under both *Frye* and *Daubert*.

While testimony by linguists has been successful in dispelling myths about language, educating judges and juries, and preventing use of language misconceptions for the securing of convictions, it is incumbent on linguistics as a field to proactively inform and educate all levels of the legal system and the forensic science community. To this end through a review of the evidentiary and procedural aspects of the United States legal system's, treatment of language evidence and the analysis of a number of recent case examples involving the application of linguistic testimony, the above points will be illustrated and provide a practical legal-scientific framework for understanding the expectations and limitations of implementing the linguistic analysis of language evidence.

**Forensic Linguistics, Expert Testimony, Authentication**

## E12 It's a Rough World Out There for Experts

*Roderick T. Kennedy, JD\*, New Mexico Court of Appeals, PO Box 2008, Santa Fe, NM 87504-2008*

The goal of this presentation is to instruct consulting and testifying experts on issues germane to the rights and responsibilities of persons involved in the litigation process. This will include relationships with hiring and opposing attorneys and the court. Conflicts of interest, contractual relationships, trial preparation, and testimony will be discussed.

This presentation will impact the forensic community by providing experts involved in difficult situations relative to litigation useful information to help them in situations where they are confronted with confounding ethical, pecuniary, and employment situations.

These issues will be discussed in the context of a fact-based scenario in which an academic researching environmental factors is solicited for expert testimony by a prominent plaintiff's lawyer in a lawsuit involving a prominent defendant. Not being familiar with litigation practices, the prospective expert witness disregarded advice to send a retainer letter setting compensation for preparation and testimony (in and out of court), and set an amount for a retainer. Instead the prospective expert proceeded on the project without receiving a retainer and, upon completion of the analysis, was fired by the plaintiff's lawyer who now also refused to pay for the expert analysis. Subsequently, the prospective expert was contacted by the defense to set up a deposition; after the plaintiff's lawyer had included the prospective expert on the list of plaintiff's witness. The prospective expert advised the defense lawyer that the expert had been fired by the plaintiff's lawyer. Concurrently, the plaintiff's lawyer sent the prospective expert a letter informing the expert that all of the expert's work in the case was the property of the plaintiff's lawyer as "work product", and that if the expert released the report or talked to anyone about the analysis the plaintiff's lawyer would destroy the expert's reputation; file a complaint with the professional licensing board and with the expert's employing academic institution; and would sue the expert

In the adversarial system, play is sometimes rough. Experts may become caught in the middle of two very committed and nasty warring sides. Not being lawyers, not all experts know their rights in difficult situations. This presentation will attempt to answer most of the questions posed by the factual situation outlined above and provide practical advice for consulting and testifying experts.

**Experts, Litigation, Testimony**

## E13 YouTube, Facebook, Chat Rooms, and Blogs: A Fertile Classroom for Illicit Activities

*Susan G. Zucker, PhD\*, National Clearinghouse for Science, Technology and the Law at Stetson University, 1401 61st Street South, Gulfport, FL 33707; and Vahid Majidi, PhD\*, Federal Bureau Investigation, 935 Pennsylvania Avenue North West, Washington, DC, DC 20535*

After attending this presentation, attendees will: (1) study the relationship between national security, criminal activity, and social networks on the internet, (2) raise awareness of the internet's impact on national security with regard to crime and terrorism, (3) comprehend the impact of internet technologies on government surveillance techniques, and (4) appreciate how technology can assist governments in apprehending criminals and terrorists online.

This presentation will impact the forensic community by raising awareness of the importance of internet surveillance to curtail criminal and terrorist activity worldwide. Attendees will see the connection between social networks on the internet and national security and criminal activity, comprehend the impact of the internet on government surveillance, and relate the development of new internet technologies with the need for improved national security surveillance techniques.

After attending this session, participants will know about current proliferation of tools, technologies, and methodologies for criminal activities on the internet. Conversely, these same tools can be used to determine the goals, methodologies, and/or intent of perpetrators and help law enforcement officials identify potential targets. This

presentation will inform and complement the theme "Future of Forensics" by discussing internet security in response to developing social networks.

In the past, complex approaches used by sophistical criminals were passed on by old fashioned apprenticeship. Interested individuals had to put themselves in jeopardy to obtain information and learn about these trades. Today, with the abundance of instruction on the internet, anyone can learn the fundamentals of sophisticated crimes at minimal risk and in nearly complete anonymity. All one needs is a computer terminal with an internet connection. For example, even Iranian president Mahmoud Ahmadinejad has his own blog.

Another unintended consequence of internet platforms such as YouTube, is the enhancement of law enforcement capabilities by examining video clips uploaded from cell phones. In fact, several investigations have been initiated based on potential volitions captured by people near a crime scene, recording ongoing activities with their cell phones. Citizens Media describes the phenomenon of people taking pictures with their cell phones and posting them on the internet, most commonly on YouTube. Two examples include the recent discovery made with the assistance of video footage of two L.A. police officers improperly treating an arrestee and a child who was filmed while supposedly being given Ecstasy.

Lastly, the content of these internet social networking sites, by themselves, can be used as potential evidence of crimes or intent. Over the past few years, local law enforcement and the U.S. government have been able to analyze the content of posted materials and arrive at attributing information yielding identification and location of perpetrators.

The speed of new technology implementation is extremely rapid and the rate of public acceptance of these new tools is well aligned with the development phase. As such, the law enforcement and U.S. government community is faced with a significant challenge when looking to use these upcoming social networking Venus. Furthermore, the technical and legal bases that were used for earlier version of deployed technologies may not be compatible with the next generation of internet infrastructure. These networks, which include Facebook, YouTube, Second Life, blogs, and secure web-based e-mail, didn't exist five years ago but are vehicles studied to identify possible illegal activities.

Wikipedia's open source technology has been used to create Intellipedia, an online system for collaborative data sharing used by the U.S. intelligence community (IC). A-Space, the web-based portal that houses Intellipedia, was created to assist the intelligence community with information sharing. It will eventually include wikis, blogs, social networking, RSS feeds, collaborative web-based word processing, mash-ups, and content tagging.

**Social Networks, FBI CIA, YouTube**

## E14 Restoration of Bodies, Retention and Disposal of Organs and Parts of Organs Removed During Forensic Autopsies: Ethical Issues Relating to the Wishes of Relatives

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After attending this presentation, attendees will be able to describe the ethical issues related to restitution of bodies, retention, and disposal of organs after autopsies. Attendees will be aware of the importance of informing families before and after forensic autopsies.

This presentation will impact the forensic science community by calling for collective reflections to determine what information should be given to families before and after autopsies, and how to modify forensic practices for improving the respect of human dignity after death.

**Background:** French law imposes strict regulations on medical autopsies (articles L1211-2 and L1232-1 to L1232-6 of the Public Health Code), requiring health professionals to inform the family and to ensure that the deceased did not express a wish during his or her lifetime not to undergo autopsy after death. The body must also be restored as closely as possible to its initial state (Article L1232-5 of the Public Health Code). However, French law provides no rules concerning the removal and disposal of organs and parts of organs during autopsy.

There is no French legislation regarding providing information to the family regarding the disposal of organs and tissues removed during forensic autopsies. It is difficult to apply the same points of law concerning medical autopsies to forensic autopsies. In the forensic context, the consent of relatives is not required and families are informed about the need for an autopsy by police officers rather than by the forensic scientist. The body is returned to the family after autopsy with the agreement of the magistrate, but there is no legal duty other than those applying to medical autopsies to make the body presentable to the family. The organs and tissues removed are sealed for evidence in judicial proceedings, but are not systematically analyzed further. They may therefore be destroyed by incineration after any analysis or if the magistrate considers them no longer relevant to the judicial proceedings.

French law seems to be lagging behind the laws of other European countries, such as the United Kingdom. After the scandals of the Isaacs case in 1987 and the Alder Hey Children's Hospital in Liverpool in 1996, British legislation was modified (Human Tissue Act in 2004). It was made mandatory to inform relatives before both medical and forensic autopsies. Pathologists and forensic scientists are required to ask relatives about what they wish to happen to the organs and parts of organs removed, which may be used for biomedical research, destroyed, or buried with the body.

Are French practices concerning the disposal and restoration of corpses, organs or parts of organs after forensic autopsies, when judicial investigations are completed really an issue? This question can be illustrated by three examples:

**Case reports:** The first example relates to what has been called "the cases of the fetuses of Saint-Vincent-de-Paul Hospital" in Paris, where hundreds of fetuses were discovered in the mortuary in August 2005. This revelation led many French people to wonder what was being hidden in mortuaries.

The second example relates to a newspaper article (Libération, 19 June 2008), which reported that a judicial inquiry had been opened to

deal with a complaint from a man who asked to see the body of his wife after a forensic autopsy. He had the unpleasant surprise of seeing the body non-sutured.  The newspaper ran the headline "When forensic medicine forgets human dignity" and the article began "It is an atrocious story ...".

The third example concerns the unusual, but legitimate request of a woman to have the organs removed from her husband during a forensic autopsy (brain and heart) restituted to her so that they could be buried with his body.  With the agreement of the magistrate, this request was granted.

**Discussion:**  These situations raise questions about our consciences and practices because there cannot be a failure to remember the changes in public opinion and attitudes that have taken place over a number of years.  Families now ask for more transparency concerning the organs or parts of organs removed, their use, and disposal.  They ask to see their loved ones and place importance on the integrity of the body after death.

**Conclusion:** Calls to inform close relatives, both before and after forensic autopsies, thus seem increasingly legitimate.  Collective reflections are required to determine what information should be given, how it should be delivered to families, and how to modify practices and to propose new solutions for improving the respect of human dignity after death.

**Medical Ethics, Autopsy, Tissue and Organ Harvesting**

## E15  We Have Lift-Off!  What Do Real Jurors Think About Trial Attorneys' Performances? What Can We Do to Lift Our Games?

*Judith Fordham, LLB\*, Murdoch University, School of Biological Sciences & Biotechnology, Murdoch, 6150, AUSTRALIA*

After attending this presentation, attendees will have acquired insights into what jurors like and loathe about the performance of trial lawyers; and with this information they will be able to improve their performances and become more effective advocates.

This presentation will impact the forensic science community by helping to improve the courtroom skills of trial attorneys who will be better able to present and challenge forensic evidence in jury trials, helping to ensure that "junk science" is kept out of court, and that legitimate forensic evidence is understood and considered by jurors in context and in proper balance with other evidence at trial.

This interactive presentation, based on detailed post-trial interviews with hundreds of jurors will inform and assist trial lawyers in presentation of their cases, and challenging their opponents, with special emphasis on presenting forensic evidence in criminal cases.   The learning gained will also apply to civil jury trials.

Both quantitative and qualitative assessments have been made by questionnaires and interviews with jurors after trials.  Some hundreds of interviews have been undertaken.  The intimidation study used a random sample of some 3,000 jurors with a 33% response rate for questionnaire completion.  Over 500 of these jurors requested to be interviewed, and are being worked through now.  An unexpected fringe benefit has been the acquisition of an enormous wealth of information volunteered by jurors in a "debriefing" situation.  Although not by definition not the focus of the studies, this information is crucial for attorneys to take into account and critically evaluate their practices simply because the jurors saw fit to volunteer it.

The presentation will include practical exercises for those brave enough to participate – although not compulsory.  It will also incorporate practical tips and suggested solutions to common jury advocacy problems. There will be time for discussion and interchange of ideas and experiences.  Forensic evidence presentation will be covered thoroughly,

but not to the exclusion of more general issues which matter to jurors.  The focus of the presentation is on practical skills, not esoteric knowledge.  Having said this, unlike much advocacy learning which is largely based on pop psychology, urban myth, and folklore, there is a solid base of social science research backing the advice and observations within this presentation.  The impact of the so-called "CSI effect" in jury deliberations and processing of evidence will be covered.

**Jury, Advocacy, Forensic**

## E16  H.R. 5843 — "Personal Use of Marijuana by Responsible Adults Act of 2008:"  A New Federal Approach to the Decriminalization of Our Most Prevalent Drug

*Heather L. Harris, JD\*, PO Box 43626, Philadelphia, PA 19106*

After attending this presentation, attendees will view the text of the proposed legislation along with the following:  pros and cons of federal legalization; state approaches to decriminalization; international approaches to decriminalization; political responses to the proposed bill; and public opinion regarding this bill.

This presentation will impact the forensic community by presenting information on the proposed bill that will allow members of the community to make informed recommendations to their respective representatives.

On April 17, 2008, Representatives Barney Frank (D-MA) and Ron Paul (R-TX) introduced H.R. 5843, the "Personal Use of Marijuana by Responsible Adults Act of 2008," a bill which would decriminalize possession of marijuana for personal use.  Although this bill is only in the first stage of becoming legislation, it is a bill that has a long and tumultuous past behind it.

This presentation will present the proposed bill in its original language.  The simple text of this bill is quite deceptive considering the complex issues swirling around it.  Public health and law enforcement issues will be addressed, government responses will be outlined, including state and international decriminalization laws, and political arguments for and against the bill will be presented alongside public opinion.

The goal of this presentation is to bring awareness to the decriminalization movement and to educate attendees so that they may come to an informed opinion on this political issue.

**Marijuana, Decriminalization, House Bill**

## E17  Deliberate or Careless Transmission of AIDS:  A Problem of Criminal Classification

*Sophie Gromb, PhD\*, Service Legal Medicine, Legale-University of Bordeaux, Bordeaux, 33076, FRANCE*

After attending this presentation, attendees will learn how the French policy intends to avoid deliberate AIDS transmission and how the prosecution is organized.

This presentation will impact the forensic science community by illustrating the difficulties in organizing prosecution against the people who purposefully transmit AIDS by not taking precautions.

Epidemiologically speaking, the first case of AIDS (Acquired Immune Deficiency Syndrome) in France dates from 1978.  Since then a public health policy has been implemented including specifically the compulsory declaration of seropositive subjects and declared cases of the disease.

Despite the noticeable drop in mortality, AIDS nonetheless remains a particularly serious transmissible disease insofar as there is still no curative treatment or any vaccine capable of limiting or stopping its spread.

For that reason, public authorities and patient associations emphasize the importance of prevention. The first way to fight AIDS and to reduce its incidence, lies in the promotion of all the individual and community measures aimed at preventing transmission or at reducing the number of incidences.

Concomitantly to the evolution of medical knowledge, there has been a change in the sociological perception and a legalization of the issue, starting with the case of contaminated blood where several thousand hemophiliacs were given transfusions with contaminated blood. Doctors, top executives, and ministers were taken to court on the charge of poisoning.

Today, the men and women who consider themselves victims of a deliberate transmission of AIDS following unprotected sexual intercourse are calling for those responsible for their contamination to be sentenced.

A review of all of the criminal qualifications applicable in such cases which make it possible to organize the repression of such behavior will be presented.

The Supreme Court has already set aside the criminal qualification and has more recently adopted the term of an offence committed through the administration of a noxious substance.

**AIDS, Transmission, Prosecution**

## F1 The Measurement of Open Apices of Third Molars to Test Chronological Age in Living Subjects Over 18-Year-Old

*Danilo De Angelis, DDS\*, Institute of Legal Medicine University of Milan, via Mangiagalli 37, Milan, 20133, ITALY; and Roberto Cameriere, PhD, Institute of Legal Medicine, University of Macerata, Italy, via don Minzoni 9, Macerata, ITALY*

After attending this presentation, attendees will be able to evaluate having arrived at a birthdate of 18 years of age in living subjects.

This presentation will impact the forensic community by illustrating a new method to determine if an individual is 18 years of age.

This paper concerns a method for assessing adult age based on the relationship between age and the third molar maturity index ($I_{3M}$) which is related to the measurement of the open apices of the third molar. Furthermore, this method was compared to those based on Demirjian's stages G and H. The sample consisted of 906 caucasian individuals aged between 14 and 23 years (53.6% females and 46.4% males). Orthopantomographs (OPGs) were analyzed by two observers and calibrated by means of the concordance correlation coefficient for the reproducibility of the third molar maturity index ($I_{3M}$) and k statistics for reproducibility of the Demirjian stages. Probabilities of an individual of being older than 18 years of age (adult age) were derived using the measurements of the third molar maturity index ($I_{3M}$). These results were exploited to set a threshold value to assign an individual to juvenile or adult age. A cut-off value of $I_{3M}=0.08$ was taken. The sensitivity of this test was 70% and specificity was 98%. Furthermore, the proportion of individuals with a correct classification was 83%. The results of the test showed a better specificity when compared to the choice of stage G and a better sensitivity when compared to the choice of stage H for adult age.

**Age Determination, Third Molar, Forensic Odontology**

## F2 Age Estimation: Aspartic Acid Racemization Utilizing Whole Teeth

*David R. Senn, DDS, 18 Villa Jardin, San Antonio, TX 78230; J. Rod McCutcheon, BS, Bexar County Medical Examiner's Office, Forensic Toxicology Lab, 7337 Louis Pasteur Drive, San Antonio, TX 78229; Paula C. Brumit, DDS, 103 East Beltline, Suite H, Cedar Hill, TX 75104; Bruce A. Schrader, DDS, 9004 Francia Trail, Austin, TX 78748; and Stefanie D. Seitz, DDS\*, 8502 Blanco Road, San Antonio, TX 78216*

After attending this presentation, attendees will be knowledgeable of a method to estimate the age of unknown decedents based on the ratio of optical isomers of aspartic acid in whole teeth or portions of whole teeth that have undergone racemization. The goal of this study is to develop a method of preparing and utilizing whole teeth for aspartic acid racemization analysis so that medical examiners and coroners can apply the method for more reliable and accurate age estimation. The age estimations using this technique may provide the forensic community with more accurate estimations and smaller age ranges to help narrow search parameters for matches between missing person and unidentified body cases.

This presentation will impact the forensic science community by providing medical examiners and coroners a method of preparing and utilizing whole teeth for aspartic acid racemization analysis for more reliable and accurate age estimation.

Age estimation using the dentition has long been useful to the forensic community. Several methods, including primary and permanent tooth formation, tooth eruption, third molar formation, and the analysis of tooth morphology including the ratio of dentin to pulp, have been utilized for age estimation. Tooth formation and development is useful and accurate for age estimation for children from before birth up to about the middle teen years. Third molar formation is only useful for estimating ages up to the late teen years; after that, the third molar is fully developed and no longer useful for age estimation. The ratio of dentin to pulp is useful but like third molar development, is subject to much variability and to fairly large age ranges. Alternatively, aspartic acid racemization offers the possibility of age estimations with smaller age ranges of +/- 3-4 years, according to some researchers.

Aspartic acid racemization is based on the natural conversion of an amino acid that is present in many metabolically inactive tissues including tooth enamel, dentin, and cementum, the lens of the eye, vertebral discs and the white matter of the brain. Aspartic acid is present at birth primarily in the levorotary or L-form. Some of the L-Aspartic Acid will spontaneously convert, or racemize, to the mirror image dextrorotary or D-form over time. Racemization is a chemical reaction influenced by various factors including pH and temperature. Of the amino acids, aspartic acid seems to racemize at a faster rate and is more useful for age estimation. Using the ratios of L- and D-forms, it may be possible to estimate the age of both living and deceased individuals within a range of +/- 3-4 years.

Previous published studies detected aspartic acid using only dentin, only enamel, or only cementum. The current project uses whole teeth or portions of whole teeth. It is considered that this will simplify the sample submission process and the pre-analysis protocol. Most earlier studies used Gas Chromatography and employed a different method of derivatization of the samples. The current project is focused upon developing and testing the most useful protocols for detecting aspartic acid from whole teeth using High Performance Liquid Chromatography and Mass Spectrometry (HPLC/MS), then quantifying and determining the ratios of the optical isomers. A database of L/D aspartic acid ratios from persons of known age is concurrently created. This database is an initial step toward the ultimate goal of gathering multiple profiles and generating data utilizing teeth from many persons of known age. A whole tooth from a person of unknown age may then be submitted, tested, and compared with the database to establish a more specific age estimation. The current status and implications of the ongoing data collection and analysis will be reported.

**Forensic Odontology, Age Estimation, Aspartic Acid Racemization**

## F3 The Use of Computer Image Recognition and Interpretation Software for Automated Dental Age Determination – A Proof of Concept

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After attending this presentation, attendees will understand why computer image recognition and interpretation software (CIRIS) have been defined as programs that allow a computer to interpret visual images and derive meaningful information from the data.

This presentation will impact the forensic community by demonstrating how the use of Computer Image Recognition and Interpretation Software (CIRIS) in forensic odontology could open the door to numerous new applications. As an unbiased and repeatable method of interpreting radiographs it will give the Department of Homeland Security a valuable new tool for rapid dental age determination. Furthermore, the ability to segment radiographs could eventually lead to automated coding of victims of a mass disaster and for more rapid method of identification by odontological means.

Computer image recognition and interpretation software (CIRIS) has been defined as programs that allow a computer to interpret visual images and derive meaningful information from the data. CIRIS systems have been utilized in a wide range of applications from automate goods inspection to autonomous navigation systems. Their use in homeland security for real-time facial recognition and automated interpretation of satellite data is well documented. Medical applications including interpretation of chest radiographs and automated mammographic mass detection are gradually entering wide spread use.

The application of CIRIS to forensic odontology has only recently begun. These systems utilize a process known as image segmentation to partition an image into multiple regions or segments based on similar values of pixels. The purpose of segmentation is to simplify the image information and more importantly to separate object from background by defining object boundaries in a process known as edge detection.

This presentation will show a proof of concept application of an image segmentation system to determine dental age based on a computer's ability to segment a panoramic radiographic image while creating biometric data of the wisdom teeth utilizing a method known as active contouring. Active contours or snakes may be regarded as autonomous processes which employ image coherence in order to identify and track various features of interest. These deformable contours have the ability to identify various object shapes within an image. Snakes have been utilized for segmentation, edge detection, and shape modeling.

The goal of the project was to attempt to identify teeth within dental radiographs and compare them to an image library. The methodology utilized in the program combines both global statistical information with local edge based information, and is thus very robust with respect to noise and initializations. Once the contours have been extracted they may be matched to a template library via various metrics in order to obtain age estimation values based on data published by Demirjian, Mincer, Senn and others.

**Computer Image Recognition, Dental Age Determination, Homeland Security**

## F4 Dental Age Estimation by Calculation of Pulp/Tooth Volume Ratios Yielded on Computerized Separation and Segmentation of Cone Beam Computed Tomography (CBCT) Images

*Hazha Star, DDS\*, Patrick W. Thevissen, DDS, Reinhilde Jacobs, PhD, and Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven, International B-3000, BELGIUM*

After attending this presentation, attendees will better understand a dental age estimation method based on the correlation between the pulp/tooth volume ratio obtained from CBCT images and chronological age.

This presentation will impact the forensic community with the demonstration of a computerized method for pulp and tooth volume calculation from CBCT images and the implementation of the attained values for dental age estimation purposes.

Dental age estimation techniques are based on the developmental, morphological, and biochemical age-related changes of teeth. Elusive and multi causal changes of the pulpodential complex decrease the volume of the pulp canal system during a lifetime. CBCT images permit a non- destructive 3D registration of teeth and afford a tool for computerized calculation of the volumes of a tooth and its pulp. The goal of this study is to develop a dental age estimation method by using the correlation between the ratio of these volumes and the chronological age of the respective examined persons.

A subset of CBCT images was collected from the CBCT dataset of the University Hospitals (Katholieke Universiteit Leuven). All images were taken with the SCANORA® 3D (SOREDEX® Finland) and exported as DICOM files. Optimal quality images of intact, maxillary and mandibular, single rooted, fully developed and pathology free teeth were selected from individuals of both sexes within an age range from 10 to 70 years. Pulp/tooth ratios of the investigated teeth are calculated after semi-automated separation and segmentation of the CBCT images in the Simplant® Pro 11.04 software (Materialize® Belgium). Statistical analysis is performed to establish the relation between the chronological age and the pulp/tooth ratio, and to test for observer bias during the semi-automated separation and segmentation procedures. All the specific results of this investigation will be reported and discussed in detail.

**Dental Age Estimation, Cone Beam Computed Tomography, Semi-Automated CBCT Separation and Segmentation**

## F5 Age Estimation by Pulp/Tooth Ratio in Canines

*Roberto Cameriere, PhD\*, Institute of Legal Medicine, University of Macerata, Italy, via don Minzoni 9, Macerata, ITALY*

After attending this presentation, attendees will see results of the research and an explanation of how the new method for age estimation works.

This presentation will impact the forensic community by showing a method for age estimation in adults.

Age estimation in adults is an important challenge both in anthropological and forensic fields and apposition of secondary dentin is

often used as an age indicator. In recent papers, *Cameriere et al.* studied the use of the pulp/tooth area ratio on canines as age indicators. The present investigation was conducted to examine the possible application of the pulp/tooth area ratio by peri-apical images as an indicator of age-at-death on a Portuguese sample. The statistical model was subsequently compared with that obtained from a study conducted on an Italian sample to establish whether a common regression model for both Italian and Portuguese samples could be developed. The Portuguese sample consists of 126 canines of males and 132 canines of females aged between 20 and 84 years. They belong to the osteological collection of Museum of Anthropology at Coimbra University. The Italian sample consists of 114 canines of males and 86 canines of females aged between 20 and 79 years and was previously analyzed in [20]. It belongs to the Frassetto osteological collection of Sassari (Sardinia) and are housed in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna. Statistical analysis was performed in order to obtain multiple regression formulae for dental age calculation, with chronological age as a dependent variable. Gender and the pulp/tooth area ratio on upper ($RA_u$) and lower canines ($RA_l$) were used as independent variables. ANCOVA analysis showed that gender did not contribute significantly compared to the variables $RA_u$ and $RA_l$. The regression model for the Portuguese sample yielded the following equations: Age=101.3 – 556.68 $RA_u$ when upper canines are considered and Age=92.37 – 492.05 $RA_l$ when lower canines are considered. Both models exhibited about 97% of total variance. The mean prediction errors were ME= 2.37 years and 2.55 years respectively. Comparisons between the previous equation referring to Portuguese sample and the equivalent linear equations proposed by *Cameriere et al.* for Italian sample did not reveal any significant differences between the linear models. These results suggested a common regression model for both Italian and Portuguese samples. The common regression models, describing age as a linear function of $RA_u$ and $RA_l$, yielded the following linear regression formulas: Age = 100.598 – 544.433 $RA_u$; Age = 91.362 – 480.901 $RA_l$. These models reflected 86% and 93% of total variance respectively. The mean prediction errors were ME= 2.68 years and 2.73 years respectively.

**Age Estimation, Forensic Odontology, Canine Pulp**

# F6     Bite Marks: Physical Properties of Ring Adhesion to Skin

*Sylvain Desranleau, DDS\*, Clinique Dentaire Desranleau Incorporated, 273 boul. Laurier, Mont St-Hilaire, J3H 3N8, CANADA; and Robert Dorion, DDS, 1 Place Ville-Marie, Suite 11238, Montreal, QE H3B 3Y1, CANADA*

After attending this presentation, attendees will acquire a better understanding of the factors influencing ring adhesion to skin.

This presentation will impact the forensic community by providing scientific evidence and evaluation of different methods for ring adhesion in addition to potentially affecting the ABFO bite mark guidelines.

A recent article[1] suggests that 87.5% of Diplomates of the American Board of Forensic Odontology excise the bite site on cadavers. It is also well documented that unsupported excised tissue may shrink by as much as 50% or more.[2] In 1981, a method was developed for ring fixation prior to tissue excision.[3] Several other methods have since been proposed to minimise tissue distortion. The scientific literature, however, reveals little supporting evidence for the preferential use of one adhesive/suturing technique over another in bite mark excision.

In August of 2007, a one week hands-on training course on bite marks was held at the "*Laboratoire de sciences judiciaires et de médecine légale*" in Montreal. This yearly session is part of an online forensic dentistry course which incorporates theory and practice leading to a certificate in forensic Odontology from the Faculty of Dentistry at Mc Gill University since 2004. During this module, the "Dorion type 5 technique" was used for pig skin excision. It incorporates TakÒ hydroplastic, mosquito fiberglass netting (screen), and cyanoacrylate gel. A new method was adopted in preparing the pig skin which involved the use of VeetÒ, a commonly used chemical depilatory. The results were disastrous; almost all of the rings separated from the skin during excision and the idea of experimenting on the physical properties of ring adhesion to skin was born.

Ring detachment can be attributed to many factors including temperature variations, ventilation, atmospheric humidity, body wetness, and temperature as well as the cyanoacrylate's physical properties not to mention other chemicals. However, little research has been accomplished to scientifically demonstrate these hypotheses as clinical experience prevailed.

The present task undertook the challenge of comparing methodologies using specific instrumentation and software used in the forensic area of ballistics with TriggerScan™ version 2.0.

The purpose of the first phase of this multi-level research is to study the measurements obtained of the tensile stress needed to rupture the bond between TAKÒ hydroplastic, the cyanoacrylate, and the pig skin. The pig skin conditions varied from untreated and hairy to shaved with and without different materials including soap, shaving cream; to treated with ethanol, VeetÒ, etc. at room temperature, with humidity/condensation/wetness removed, and with "fresh" cyanoacrylate glue versus gel. The results give a clearer scientific exposé of the physical properties of the various materials utilized and their interaction.

In conclusion, by compiling and analyzing the precise measurements, risks of tissue distortion during bite mark excision could be significantly reduced by utilizing recommended techniques and materials which could ultimately facilitate perpetrator identity.

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1, 2 Tissue Specimens: Invasive Analysis; Bite mark Evidence, Dorion RBJ, ed., Marcel Dekker (CRC Press), New York, NY, 2005; 228-29.

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**Bite Marks, Ring Adhesion to Skin, TriggerScan™**

## F7 Three-Dimensional Analysis and Comparison of Human Anterior Teeth and Experimentally Created Bite Mark Depressions

*Cristina M. Dalle Grave, DDS\*, PO Box 986, Angel Fire, NM 87710; Alex A. Da Luz Dos Santos, BCS\*, Angel Fire Resort, 35 Vail Avenue, Unit 201, Angel Fire, NM 87710; Paula C. Brumit, DDS, 103 East Beltline, Suite H, Cedar Hill, TX 75104; Bruce A. Schrader, DDS, 9004 Francia Trail, Austin, TX 78748; and David R. Senn, DDS, 18 Villa Jardin, San Antonio, TX 78230-2749*

The objectives of this presentation are to: (1) describe a method of digitizing three dimensional contours of human anterior teeth from dental casts (models) and the depressions in leather experimentally created by those teeth, (2) describe the development and use of specialized software to accomplish metric and pattern analysis when comparing the two sets of data, and (3) discuss the statistical analysis of the comparisons generated by the software.

This presentation will impact the forensic community by creating a method of comparing bite mark depressions to dental casts in three-dimensions instead of the more common two-dimensional methods.

**Hypothesis:** The MicroScribe 3D Digitizer facilitates the recording of accurate three-dimensional data information from both teeth and the marks made with those teeth biting into an analogue of human skin. The data recorded from dental casts and the depressions created in the skin analogue can be analyzed and processed to create three-dimensional dental profiles for the anterior teeth of that person and for the depressions in the skin analogue. The two sets of data can be critically compared by special software that facilitates metric and pattern analysis.

**Background:** Johnson et al at Marquette University have proposed a system to scan dental models to record two-dimensional and three-dimensional features seen in the anterior teeth. This information can theoretically be used to create a database of dental profiles.

Tooth depressions in human skin occur at bite infliction and remain for varying periods of time when the person bitten is living. In the living the marks may fade, disappear altogether, or become raised in an inflammatory response. In deceased individuals the tooth indentations in skin may remain until decomposition.

In the cases where the depressions made by teeth remain, a model of the bite mark impression can be very useful for analysis. The resulting model shows the curvature of the surface bitten, that may not be apparent in two-dimensional photographs. Dorion (2005) stated that the process of creating a 2D image from a 3D object leads to loss of information.

**Materials and Methods:** Dental casts mounted on Hanau articulators were randomly selected from a New Mexico population of individuals between the ages of 20 and 50 years. Cowhide leather with a single layer thickness of approximately 0.5cm was doubled to simulate the folding of skin caught between upper and lower teeth in some bite marks scenarios. The leather was wetted by soaking in water and pressure was applied using the Reynolds Controlled Bite Force Generator (RCBFG), a device. The Reid Bite Reader (RBR) was used to measure the bite forces generated and to calibrate the RCBFG setting required to consistently apply forces that created the teeth impressions into the leather.

Using the Immersion MicroScribe 3D Model G2X Digitizer, information from both the bite mark depressions and the dental casts was transferred to a computer using auto scan properties. The auto scan was set to capture points at 0.5mm intervals. A systematic method for digitizing from the first pre-molar to the opposite first pre-molar was developed.

A sample of 50 bite mark impressions and 50 sets of dental casts were digitized. The patterned injury tooth depression datasets were entered as the unknowns and the dental cast datasets as the knowns by the use of unique numbers into the database.

The developed software performed a 3D comparison using metric and pattern analysis. Selected XYZ axis points recorded for each individual tooth and each tooth depression in the bitten substrate were analyzed. The analysis images were rotated into various orientations for viewing and to facilitate analysis. The software compared any individual point with another individual point and varying combination of points. A threshold setting was applied in the software to allow the display of all of the points, the most prominent points, and various intermediate settings.

The software generated a report quantifying the statistical similarity of the selected points. Indices of similarity were developed to indicate the likelihood that tooth depression data and dental cast data are the result of a cause-effect event. The dental data from the various models was also compared to generate an index of similarity between different data sets of teeth. This last feature may be used to augment, support, or critically examine research into the uniqueness of the anterior human dentition.

**Results:** The features of the incisal and occlusal surfaces from the dental casts were transferred to the wet leather. The MicroScribe 3D Digitizer is capable of recording accurate three-dimensional information from both depressions created by teeth and the teeth that may have created those depressions.

Blind testing with the bite mark and dental cast databases eliminates some forms of expectation bias which is very important in evidence based studies. The specificity and sensitivity was determined by statistical analysis and were expressed in a ROC curve.

The new software facilitated precise metric and pattern analyses that were valid for comparing two sets of data. To evaluate the reliability of the software developed for this pilot study, larger sample tests and clinical trials must be performed.

**Forensic Odontology, Bite Mark, Three-Dimensional Analysis**

## F8 Forensic Odontologists' Armamentarium for Dental Identification in a Rural Setting

*Pamela Jurgens-Toepke, DDS\*, University of Illinois College of Dentistry, 801 South Paulina, Chicago, IL 60612*

After attending this presentation, attendees gain the knowledge to construct a functional, well equipped portable tote that will give him/her the ability to perform forensic identification in a rural Coroners office.

This presentation will impact the forensic community by helping the neophyte rural forensic odontologist to compile a well equipped portable tote that contains commonly used implements for performing a dental identification.

At the conclusion of this abstract, the forensic odontologist will have the knowledge to construct a functional, well equipped portable

tote that will give him/her the ability to perform forensic identification in a rural coroner's office.

Small communities often have a small coroner's office that may service several counties. The crime rate is usually lower in a small rural town. Cases requiring a forensic odontologists' expertise occur seldom in small rural towns and counties. Small towns often have limited resources. Odontologists may be responsible for their own equipment when assisting in the identification of an unknown victim. The following is a list of tools, forms, and photographic equipment that should be standard fare for the rural forensic odontologist: mouth mirror, explorer, cotton forceps, scaler, toothbrush, scalpel, rope wax, gloves, mask, disposable lab jacket, and extra lighting such as a headlamp. A headlamp, commonly used for camping is great for hands free extra light. Employing a UV LED light illuminate composites, and make them readily detectable. Scalers and toothbrushes clean off dried blood, tissue, and debris from the teeth. Blood and tissue are often encrusted onto the tooth surfaces and need to be removed so that the teeth and their restorations can be evaluated. Rope wax can be used to hold an x-ray film next to the teeth or for propping up jaw fragments on a table for radiographing. Jaw dissection is necessary when the body is severely burned, decomposed or in rigor, prohibiting the odontologist from opening and examining the jaws. A *Striker* saw is commonly used to cut and dissect the maxilla and mandible. Most coroner's offices have these saws available for the pathologist. If a *Striker* saw is not available to dissect the jaws, a *Sawzall* can be employed. A *Sawzall* is a reciprocating saw, commonly used for home construction and remodeling. It is approximately 18 ¾" in length and weighs around 8lbs. The ½ inch blades can be replaced as needed. The 15 amp *Sawzall* is the most powerful version and can be purchased at a hardware store. Garden loppers can be used to cut through the ramus of the mandible. Lopper handles come in a variety of lengths. The standard lengths are 26-37 inches with a 3 inch cutting diameter. Longer handles afford the operator more leverage. The blades can be cleaned and sharpened. Once the mandible is removed, the removal of the maxilla is not always necessary. When the mandible is removed, the maxilla is visualized. Postmortem, antemortem dental charts, ABFO (American Board of Forensic Odontologists) ruler, pens, pencils, and radiographic mounts should be part of the forensic odontologists armarmentarium. Standard dental charts can be employed or copies of antemortem and postmortem charts can be copied from a variety of forensic books and manuals. If a dental xray unit is available, periapical radiographs should be taken of the full mouth or whatever fragments are available. If the body will be viewed at a wake, then the jaws should not be dissected. A skull xray can be ordered from the local hospital or through the morgue facilities. Periapical radiographs are more diagnostic than a skull radiograph. Superimposition of the right and left sides of the jaws occur when full skull radiographs are taken. Intraoral cameras are used to photograph the victims' teeth, and also alleged bite marks. The ABFO ruler should be in all bite mark photos along with the case number. Digital cameras are ideal for downloading the images to a computer. The *ADA Professional Product Review* stated that some dentists have found that Acclaim USB, CDR USBCam2, and SOPOR 717 produce high quality images. *WIN-ID* computer program is ideal for mass disasters. Antemortem and postmortem dental charting can be entered into this program and then a comparison is run by the program to match the records. Forensic odontologists need to carry their own equipment when assisting small rural coroners.

**Armamentarium, Rural, Odontologist**

## F9 The Vampire Bites Back in Odontology and Anthropology: Case Report of Skeletal Remains in Nuovo Lazzaretto Island, Venice

*Emilio Nuzzolese, DDS, PhD\*, Ambulatorio Odontoiatrico Nuzzolese, viale J.F. Kennedy 77, Bari, 70124, ITALY; and Matteo Borrini, BA, via del Mattone 17, Cadimare (SP), 19131, ITALY*

After attending this presentation, attendees will learn about one of few cases where a presumed vampire is analyzed from an odontological and radiological perspective.

This presentation will impact the forensic community by showing the importance of a taphonomic profile for forensic assessment.

Since the summer of 2006, the Archaeological Superintendent of Veneto (Italy) has promoted research on ancient mass graves located on Nuovo Lazzaretto Island in Venice. During the searches a large number of fragmented and commingled human bones were found. The burials were at different stages and are believed to be the remains of plague victims from numerous outbreaks of pestilence which occurred between the fifteenth and seventeenth centuries.

Among the remains, an unusual burial was found. The body was laid supine, with the top half of the thorax intact, arms parallel to the rachis axis, the articulations were anatomically unaltered. Both the skull morphology and the dimensions of the *caput omeris* suggest the body was female. A brick of moderate size was found inside the oral cavity, keeping the mandible wide open.

Data collected by the anthropologist was used to generate a taphonomic profile, which precluded the positioning of the brick being accidental. Likewise, the probability of the brick having come from the surrounding burial sediment was rejected, as the only other inclusions found were bone fragments from previous burials in the same area. The forensic profile was based conceptually on the "circumstances of death" and concluded that the positioning of the brick was intentional, and attributed to a symbolic burial ritual. This ritual confirms the intimate belief held at those times, between the plague and the mythological character of the vampire.

Vampires, or the "un-dead", were in fact considered to be the cause of the pestilence and during the exorcism it was usual to insert something into the mouth to prevent mastication. Such individuals were interred with the sudarium removed, and the mouth filled with a handful of earth, a stone, or brick.

The taphonomic profile is presented together with the results of the odontological and radiological analysis of the jaws and tooth fragments.

This case could well be the first "vampire" burial archeologically attested with medico-legal, and forensic odontological analysis.

**Cranial Trauma, Forensic Taphonomy, Dental Radiology**

## F10 An Alphabet Soup of Dental Databases

*Sheila Dashkow, DDS\*, 7675 Maple Avenue, Pennsauken, NJ 08109; and Donna A. Fontana, MS, New Jersey State Police, Office of Forensic Sciences, Forensic Anthropology Laboratory, 1200 Negron Drive, Hamilton, NJ 08691*

After attending this presentation, attendees will become familiar with the dental community, as well as with the multiple dental databases used in the recording of teeth and their restorations.

This presentation will impact the forensic science community by displaying the importance and necessity of using a trained forensic odontologist in the dental database documentation of unidentified and missing persons.

The comparison of a missing person's dental records, models, and radiographs with the dental evidence from unknown remains has stood the test of time as a means of positive scientific identification. Antemortem dental records and dental x-rays are utilized on a daily basis in the identification of unidentified human remains. These records are obtained, generally by law enforcement from the family dentist of the missing individual. In the United States, general dentists utilize the Universal Coding System in their practices. Each general dentist also has their own short hand or abbreviations which they use to describe the procedures that they perform on a routine basis. For proper entry of this information, a forensic odontologist, who is knowledgeable in the varied nomenclature used by dental databases as well as the current dental terminology used today, must correctly interpret the dental records.

At the present time, there are three different dental databases in use for the documentation of dental records. These include the National Crime Information Center (N.C.I.C.), National Missing and Unidentified Persons System (NamUs), and WinID. Law enforcement has been tasked to ensure that entries are made into N.C.I.C. Medical examiners are encouraged to ensure that all unidentified remains are entered into NamUs. Forensic odontologists using the latest dental technology use WinID when charting dental information. WinID has a function to translate its codes into the N.C.I.C. format. Each database has a similar but unique coding system. Entries are made into each database using specific letters to represent a unique dental term. The letter code in one system does not always translate exactly into another system. There is even a difference in some of the symbols used for missing and unidentified remains in one of the systems. These multiple coding systems challenge the forensic community handling unidentified and missing persons to ensure the accuracy of the entries. In this regard, it is imperative that a forensic odontologist trained and familiar with each of these coding systems be involved and responsible for accurate data entry and verification in the different databases.

In order to demonstrate the differences between dental databases, a case study will be presented showing how one individual is coded using each of the different databases. Based on an actual case, a missing person's unique dentition presents a challenge to the forensic odontologist to properly enter information in each system. Some features in this case such as veneers are considered to be routine dental care. Some features such as the number and position of teeth are truly distinctive.

Careful documentation of dental information by trained forensic odontologists would not only ensure accurate dental coding but would increase the number of individuals who could be identified.

**Forensic Odontology, N.C.I.C., NamUs**

## F11 A Study of Familial Bite Marks: Can We Discern Uniqueness?

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After attending this presentation, attendees will investigate whether familial dentitions that share common hereditary alignment patterns can be distinguished following production of bite marks in human cadaver skin. Attendees will appreciate how the distortion inherent in a bite mark on skin can diffuse differences that are apparent by metric analysis of the dentition itself.

This presentation will impact the forensic community by simulating a closed population of potential biters who have genetically related tooth alignment patterns.

Frequently the forensic odontologist is consulted to determine if pattern injuries on infants and young children can be attributed to a family member. Young children, specifically infants, are generally cared for by a small circle of relatives. The caregiver group is most often a closed (limited) population and may even include three generations from a single family. In such cases the forensic odontologist is directed to perform a complete analysis and documentation of the bite mark. After excluding the possibility of a self inflicted bite mark, a comparison must then be made against the dentitions of this closed group.

It is well known that physical characteristics are inherited from parent to child. Hair and eye color, stature and facial structure are obvious examples, but this also extends to dental alignment patterns. In situations in which there has been no orthodontic intervention, there may be similarities between the dentitions of parent and child that are recognizable and distinct from the general population.

In this study, impressions were collected from varied family groupings. Models were made of these impressions using dental stone. Hollow volume overlays were produced from the models using the Johansen and Bowers method. Metric analysis was performed on the overlays of each familial set of dentitions and similarities in metric dimensions and tooth angulations were noted. The models were then mounted on vise grips that served as a biting apparatus.

Human Subject Review Board (HSRB) exemption was applied for and granted for cadaver use in this project. The cadavers were obtained following rigor mortis, were stored at 4 degrees C, and were un-embalmed. The use of cadavers for such studies has been demonstrated to be the closest model to living human skin. Bite marks were then produced in cadaver skin using the familial models. As far as possible a similar anatomical location was used for the bite sequences. The bite marks were photographed immediately following the bite. The photographs were sized 1:1 with the overlays and comparison of the overlays to the bite mark was performed.

The first goal of this experiment was to determine if familial patterns could be recognized in the dentition. The second goal was to ascertain whether the similarities noted were sufficient to complete bite mark analysis once the bite pattern was recorded in skin.

Familial similarity in dental alignment is potentially an issue that the forensic odontologist should be concerned with in closed population incidents. This study seeks to gain an understanding of the impact of this issue in bite mark analysis.

**Bite Marks, Pattern Injuries, Heredity**

## F12 The Response of Skin to Applied Stress: The Influence of Force per Unit Area in Bite Mark Analysis

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After attending this presentation, attendees will see the response of skin to applied stress during bite mark infliction.

This presentation will impact the forensic community by investigating how skin deforms during application of stress, allowing for appreciation of distortional properties of skin.

Distortion is inevitable in a bite mark. Knowledge of how distortion arises is important for the forensic odontologist. How skin deforms in response to the applied stress of a bite is dictated by the biomechanical properties of skin coupled with the 3-dimensional properties of the skin and teeth.

There are many factors that influence how a bite distorts the skin. They can be summarized into two main categories: those associated with the biter and those associated with the victim. Some of the variables associated with the biter include maximum anterior bite force, tooth arrangement, sharpness, and the manner at which the bite is made. These variables can be controlled in an experimental situation.

The more complicated set of variables are associated with the victim, mainly the biomechanical properties of the skin and underlying substrate. Skin is complex due to its non-linear behavior in response to stress. Stress is the force per unit area of a material in response to a load. Stress is generated in skin from the bite pressure on individual teeth causing the skin to go into tension. In skin, the level of stress generated from a bite is directly related to the applied bite pressure, the rate of application, percent elongation, and the rate at which the supporting tissue dissipates the load. These factors will directly influence when the skin reaches its elastic limit.

As skin strains, its properties change. At low stresses, the skin is fairly elastic. As stress increases, the skin becomes viscous, hence causing the skin to stiffen. When the skin stiffens, further elongation is limited. Since stress is expressed as force per unit area, as the contact area of the dentition is reduced, stress applied locally to the skin increases. Given the same biting force, a dentition with fewer teeth will inflict more stress on the skin. This property has the possibility of influencing the appearance of the dentition once impressed on skin.

Human Subject Review Board exemption was granted for this project. Bites were inflicted on unembalmed cadavers after the passage of rigor mortis on naked skin. The cadavers were stored at 4ºC, allowed to warm to room temperature and any condensation on the skin was removed.

Polyvinylsiloxane (PVS) impressions were taken of an individual with an average class I dentition who served as the biter. The PVS impressions, upper and lower dentition, were poured under vacuum in low viscosity metallographic epoxy resin.

Multiple sets of epoxy models of the biter were created. One set had a complete dentition. In the other sets, the teeth were systematically removed in order to vary the contact surface area.

A custom biting apparatus was fabricated. This device articulated the teeth into maximum intercuspation. The maxillary member had an integrated force transducer to allow for constant monitoring of the applied bite force. The bite force was generated by a clamping mechanism to provide for a steady, controlled application of bite force. The force transducer was connected by USB cable to a PC and the controlling software allowed visualization of the force application rate and maximum force attained.

The results were photographed with an ABFO ruler in place and the distortion was assessed. This was accomplished via metric/angular measurements (Johansen and Bowers method) and hollow volume overlay comparison (Johansen and Bowers). Each photo was sized 1:1 and analyzed with image editing software. Each set of models was scanned on a flatbed scanner, sized 1:1, hollow volume overlays were constructed and metric/angular measurements were again obtained. The area of the biting dentition was calculated with image analysis software.

Altering one single dentition allowed for comparative study of how the force per unit area (stress) relates to a bite mark, as well as how the biomechanical properties of skin alter at the moment of tooth contact, thus allowing investigation of how distortion arises. This allows for an understanding of the dynamics of the juxtaposition of the dentition with the skin in bite mark analysis.

**Bite Marks, Bite Mark Research, Skin**

## F13 The Relationship of Uniqueness and Resolution in Bite Mark Analysis

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After attending this presentation, attendees will know the extent of resolution loss of the human dentition once it is impressed in human skin.

This will impact the forensic community by demonstrating the limitations of skin as a recording medium and the tools used to record this transference.

Bite mark analysis may be simply described as the comparison of the dentition to a bite mark, both of which are in the form of a dataset, whether it be a photograph, scanned overlay, or 3D-dataset. With each of these means of recording the subjects there is an inherent transform factor that alters the data in some way.

The assumption behind this is that skin itself records the features of the dentition. While it has been stated that skin is a poor recording medium, many of the boundaries and limiting factors of this statement have not been clearly defined.

With a recording medium such as photographic film, the smallest object that can be resolved is determined by the camera's optics and ultimately by the grain size of the photographic emulsion. With digital recording devices the resolution is similarly dictated by camera optics but also by the physical size of each pixel sensor (not the total number of pixels). While the spatial resolution of photography is good, the limiting transform of photography in bite mark analysis is that the resulting record is two-dimensional.

The advent of 3D laser scanners is promising, especially in the presentation of data to a court, but costs can be prohibitive for high resolution scans. The transform in these datasets is in post-collection processing including data fusion and smoothing.

In bite mark analysis, the current and most often used method of comparing suspect dentition to bite mark is to generate a hollow volume overlay of stone dental casts using a flatbed scanner and image editing software. If the scanner is set to 300dpi, then each pixel has a dimension of 85x85 microns, therefore the smallest object that can be distinguished in the resulting image is 85 microns. If a line of single pixel width is used to create the overlay, then the human eye can readily resolve and follow the changing positions of the line that delineates the overlay. The human eye can typically resolve 80-micron particles, which enables us to visualize small detail.

The observer can resolve small differences by simple visual inspection of overlays from similar dentitions. Human pattern recognition capabilities enable us to conclude that the dentition is unique to at least a resolution of 85 microns. This conclusion, based on our visual acuity, supports the intuitive premise that the dentition is unique. However, a number of factors in the scanning process determine the boundaries of the overlay lines. There are transform factors inherent in the process of creating an overlay that effectively reduce the resolution of the representative dentition.

In a bite mark, the defining edges of the bite pattern are much more difficult to identify, even with clear indentations. This is further complicated by the fact that there is inevitable distortion due to the visco-elastic properties of skin and the 3-dimensional aspects of the teeth and skin. This second transform factor due to the skin can be greater than that of the scanning process and the two factors are additive, combining to reduce the effectiveness of the comparison. Thus when superimposing an overlay on a bite mark photograph the operator must mentally apply an arbitrary distortion correction in order to 'match' the dentition with the bite.

Human Subject Review Board exemption was granted for this project. In the course of performing bite mark research using human cadaver skin as the recording medium, situations were created which demonstrate the concepts stated above. This will be illustrated through comparisons of suspect dentitions and bite marks.

A loss of resolution on a millimeter scale can be anticipated once the representation of the dentition is transferred to skin. Under these circumstances, the level of uniqueness or resolution of measurement of the dentition (85 microns in this example) becomes tested. This prompts a reexamination of the oft-stated dual assumptions that the representative dental cast is unique and that that uniqueness is transferred to skin.

**Bite Marks, Uniqueness, Resolution**

## F14 Use of 3-D Imaging and Mathematics in Assigning the Probability of a Match Between a Dental Model and a Bite Mark

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After attending this presentation, attendees will be briefed on a way to determine an objectively-based probability of a match of a bite mark to a 3-D dental model.

This presentation will impact the forensic community by serving as a key aspect of forensic odontology methods for identification based on bite marks.

The process anticipated with this method is for the forensic odontologists to prepare dental molds of the mouth of the suspect and perform a physiological analysis based on observation and normal 2D photography. In cases where it is believed that a match has been found, and in which the bite mark has some definition, an additional test can be conducted to estimate the probability that the proposed identification is warranted. This is where the methods described in the current paper come into the process.

Using a suitable 3D camera, a computer-resident model is made of the suspect's teeth. At first the model comprises several partial representations of the full 3D object. Afterwards the separate partial models are stitched together to give a full model of the top and all sides of the model. A 2D rendition of the bite mark is also transferred to the computer. The computer is then used to calculate the optimal alignment of the bite marks to a cross section of the 3D dental model based on a "distance" measurement which is a goodness of fit measure. The process repetitively intersects the model with a plane at different depths, angles of attack, and angles of rotation calculating the distance in each instance. Optimality is defined by a distance measure that is minimized over all possible cross-sections as well as all possible rigid 2D alignments (translations and rotations) of the 2D bite marks contours and 2D cross-sections from the 3D model. The distance measure is then used to estimate the probability distribution using a logistical model. The end result is an estimate of the probability that the given teeth/mouth (as represented by the dental mold) could have made the subject bite mark.

For this early phase study, dental molds of unknown "suspects" were used. Artificial bite marks were created and photographed. These images were then intentionally distorted digitally to represent various levels of clarity typically seen in bite marks. The methods used to create the images and collect the data will be described. In addition, the methods of 2D-3D bite mark matching will be developed and detailed. The statistical analytical techniques used in computing the probability of correct match will be described and specified.

Finally data will be shown comparing the findings of forensic odontologists and the computed probabilities. In 88% of the cases the computed data was correct in assigning a relatively high probability. Only one situation resulted in a bad assignment and that has been traced to the way the distance measurement was made. Other than this

instance, the analytical methodology worked well and it is reasonable to expect that such a process could be addend to bite mark evaluations to increase their credibility to juries.

This was an early phase study and as a result, most of the effort went towards developing the methodology. Only a limited sample of molds and bite marks were available. It also relied on artificially produced bite marks. Future work should include larger samples and actual bite marks. Further work should also address improving the distance measurements.

**Bite Mark Identification, Odontology, Probability**

## F15 Role of Bite Mark Analysis in the Judicial Investigation of an Attempt to Commit Manslaughter: A Case Report

*Patrick W. Thevissen, DDS\*, and Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven, 3000, BELGIUM*

After attending this presentation, attendees will be informed about the significance and the crucial consequences a bite mark examination can have during a criminal investigation.

This presentation will impact the forensic community by demonstrating the importance of utilizing bite mark guidelines of scientific standard setting organizations during every step of the evidence collection, analysis, and comparison of the evidence and the reporting procedure within a bite mark investigation.

Bite marks on human skin surfaces are patterned injuries remaining after the displacement of soft skin tissues by the hard tooth materials during biting. The dermal pattern that remains is a reactive response of the injured skin on a variety of factors depending on the victim, the biter, all the involved circumstances during the biting act, and the moment of registration. Appropriate scientific examination and analysis of provided and gathered bite mark evidence can afford information putting a criminal investigation on the right track.

In this presentation a soft tissue injury was found on the inner side of the left arm of a woman, a victim of an attempt to commit manslaughter. The victim declared that during the offense the suspect bit on her arm while she was grabbing and holding off a knife clenched in his fist. The suspect proclaimed not to have bitten firmly.

Almost eleven months after the facts, the investigation judge requested a bite mark investigation, asking if the soft tissue injury was a bite mark and if the bite was inflicted by the victim or by the suspect. The presented bite mark investigation had to be performed on bite mark photographs taken by a medical examiner the day after the crime event. After signing a declaration of informed consent, a collection of dental evidence on the victim and suspect was carried out by the appointed odontologists. All the collected information was analyzed and blinded to compare to a line- up of tracings following diverse methods by the two forensic investigators separately. Before reporting the opinion, consultations with other forensic odontologists were taken into consideration.

It was found that the inflicted injury was a human bite mark and with a high degree of certainty the victim could be excluded. The suspect retained was the possible biter. The methodology followed during this bite mark investigation will be demonstrated in the presentation.

This case highlights the importance of the recognition of skin injuries alleged to be caused by human teeth and the immediate consult

by forensic odontologists familiar with this specific investigation for correct evidence collection, analysis, and comparison. The results obtained in this case provided the crime investigating authorities new evidence of probative value.

**Forensic Odontology, Bite Mark Investigation, Criminal Investigation**

## F16 Anatomy of a Brady Violation

*Roger D. Metcalf, DDS\*, Tarrant County Medical Examiner's District, 200 Feliks Gwozdz Place, Human Identification Lab, Fort Worth, TX 76104-4914; and Robert G. Williams, DDS, 11661 Preston Road, Suite 141, Dallas, TX 75230*

After attending this presentation, attendees will see that Brady violations can and do occur, and offer a cautionary tale for the expert witness "caught in the middle" between the defense and prosecution.

This presentation will impact the forensic community by helping the expert witness avoid being entangled in a potential Brady violation (non-disclosure of exculaptory evidence).

In *Brady v. Maryland*, 373 U.S. 83 (1963), the U.S. Supreme Court held that *exculpatory evidence* (i.e., evidence favorable to the defendant) developed by the prosecution *must* be disclosed to the defense, **if** defense counsel asked for it. A "Brady violation" is a violation of this right to exculpatory evidence under the United States Constitution.

Some federal District Courts now require automatic disclosure of exculpatory evidence; some District Courts, on the other hand, do not require disclosure of Brady evidence if the defendant, using reasonable diligence, could have developed the evidence himself. Through case law, this holding has been further extended to require law enforcement officers, as well, to disclose exculpatory evidence to the defense. Many federal District Courts require Brady evidence be disclosed to defense counsel within some specific period of time ranging between at the time of arraignment up to within 28 days after the arraignment hearing. Conversely, some federal District Courts hold that the exculpatory evidence should be disclosed "...as soon as possible…" or "…before the trial…" The Texas statute does not require automatic disclosure of exculpatory evidence, and, further, the defendant "must show good cause" for discovery of such material; but there does not seem to be a specific time limit on when defense may ask for disclosure of exculpatory material.

No federal District Court has a specific remedy for a Brady violation—sanctions are fully within the discretion of the Court. Most District Courts allow sanctions for both parties for general discovery abuses. These sanctions may include exclusion of evidence at trial, a finding of contempt, granting of a continuance, and even dismissal of the indictment with prejudice. Texas statute generally, though, does not allow the harsh remedy of dismissal to remedy a Brady violation.

In this instance, expert witnesses called by the prosecution developed potentially exculpatory evidence during the course of a bite mark analysis in a Texas capital-murder case. Through no fault of the witnesses, and through no willful, intentional, or egregious act of the prosecutor, this evidence was not transmitted to defense counsel in a timely manner. When defense counsel subpoenaed evidence from the prosecution experts (only 10 days prior to the trial!) and the evidence was sent to the defense expert, the defense expert quickly realized that defense counsel was not aware of all potential suspects in the case. One of these suspects had *not* been excluded as a potential "biter" by one of the prosecution's experts. In this case, the Court granted a lengthy

continuance to allow the defense expert adequate time to perform his own thorough bite mark analysis.

**Brady, Exculpatory, Evidence**

## F17 A First Bite Mark Case

*Richard H. Fixott, DDS*, 6690 Southwest McVey Avenue, Redmond, OR 97756*

After attending this presentation, attendees will be shown how to apply bite mark training to prepare for a case and to review basic principles of bite mark analysis.

This presentation will impact the forensic community by providing an example of how to apply training to forensic odontology.

After several exciting AAFS or ASFO meetings, many become frustrated by the lack of opportunity to use what is learned. This presentation is an example of how one may use knowledge and skills acquired during these meetings. My first bite mark case was a fabricated exercise. A bite was made, examined, photographed, and preserved. All suspects with access were located and evidence obtained. An initial comparison based on general characteristics allowed exclusion of one suspect. A more detailed analysis using individual characteristics revealed good concordance with one suspect and poor concordance with another. Faced with the comparison, the culprit confessed.

This bite mark comparison has been used effectively in presenting the principles of bite mark analysis to both professional and lay audiences. The presentation reviews gathering of evidence, preservation of evidence, and different methods of comparison. Working with the legal system can also be discussed. The case also provides an opportunity to use and practice techniques learned during courses and articles devoted to bite mark evidence.

**Bite Mark, Forensic Odontology, Dentistry**

## F18 "With Friends Like These..." Revisiting a Homicide Case Replete With a Bite Mark

*Bruce R. Wiley, DMD*, Big Horn County Sheriff Department, 337 Greybull Avenue, PO Box 206, Greybull, WY 82426; and Robert C. Byrd, DDS, PO Box 6789, Sheridan, WY 82801-7106*

After attending this presentation, attendees will recognize the importance of striving for perfection while gathering evidence in a bite mark case. Attendees will also be introduced to an example of a homicide case where the forensic odontologist might have been utilized for more than merely identifying the perpetrator of the bite injury, as was done in these proceedings.

This presentation will impact the forensic science community by illuminating the fact that the forensic odontologist can be an important adjunct of the crime scene investigation team.

Locating elk hunting camps in Wyoming is amongst the most sacred of traditions for families, especially in the Big Horn Mountains. Generation after generation has laid claim to and improved upon the location of their family hunting camp, much as their ancestors had "proven up" their familial homestead. Families and friends have thrived in their celebrated, and much anticipated, annual treks up the sides of the mountains to collect their game for the winter pantries; and several have perished, never to return.

David Lawrence Palan and Michael Thomas Fleming fast became best friends when they first met while attending the same elementary school in the second grade. For over thirty years, their families had grown close as well, with hunting being only one of the many activities in which they shared and unified their friendship. They had staked their claim to the location of their camp many years prior to this noteworthy hunting season and this particular hunting episode could well split the families apart.

In October 1996 the annual ceremonial of pitching camp, consuming vast quantities of beer, and surveying their assigned hunting grounds for any and all signs of wildlife had commenced. Many hopeful hunter friends attended during the previous days to erect the encampment and had since returned home. These other eager game seekers had employment priorities and awaited their time away from their employment so that the hunting could be enjoyed at a later date.

On the evening of October 19, 1996, David Palan, Michael Fleming, and Frank Fleming, Michael's father, happened to pass each other on the road leading to camp. Mr. Fleming was headed up to camp after acquiring provisions in town. David and Michael were headed down to the Hyattville Bar at the bottom of the mountain for a beer and to watch an exotic dancer perform. Dad later testified that they had left in good spirits.

Apparently, the combination of spirits imbibed at the local watering hole and harsh words toward each other led to successive altercations back at camp. Although Frank Fleming was sleeping in a camper adjacent the camp tent, he reported hearing shouting by both David and Michael, upon their return. Mr. Fleming stated that he went back to sleep, concerned about the few short hours of sleep that remained prior to awakening for their first day of hunting.

Apparently, the boys were only slightly inebriated, but their derogatory insults and constant verbal chastising led to an initial wrestling match outside the tent in the cold snow. David Palan testified that he had Michael Fleming in a "scissors hold." Fleming reportedly tried "gouging out" Palan's eyes, so Palan bit Fleming on the arm.

Tired and cold, the two returned to the tent to stoke the fire and warm themselves. The verbal banter recurred while sitting next to the woodstove. During the second wrangling a new .45 Ruger Blackhawk single action revolver, reportedly securely holstered on David Palan's right hip, was somehow cocked and discharged. Michael Fleming caught the bullet slightly above the left eye and was mortally wounded.

This case will be presented to expose the necessity of precision in evidence gathering should the investigator be called to testify. In addition, the investigators of this incident might have chosen to use the forensic odontologist in interpreting the bite mark evidence in relation to the potential crime. In this case, the odontologist was engaged primarily to identify the perpetrator of the bite.

**Bite Mark, Homicide, Investigation**

## F19 The Forensic Dental Articulator in the Identification of Human Bite Marks

*William Pace, DDS*, 344 - 15th Street, West Babylon, NY 11704*

After attending this presentation, attendees will be made aware that when a bite mark is inflicted, the maxillary or upper jaw, is related anatomically to the lower jaw or mandible. Due to different sizes and shapes of individual temporal-mandibular joints and jaw structure, this affects the gross dimensions of the bite mark pattern.

This presentation will impact the forensic science community by adding techniques in the identification or exclusion of suspects in bite mark cases where DNA is absent or a few teeth are present in each jaw.

A forensic dental articulator is capable of detecting that anatomical relationship. This new technique adds one more dimension in the bite mark analysis. At present in bite mark analysis only the teeth in one or both jaws are related to life size photos of the bite mark, in order to either identify or exclude a subject.

Dental articulators have been used in dentistry to relate the maxillary and mandibular jaws for the examination, diagnosis and treatment of various dental disorders. A wax bite is taken on the patient in what is called centric relation, which gives the anatomical relation of the teeth when the mandibular condyles are allowed to move in the maxillary fossa in the most distal and superior position, until the teeth make first contact. A clinician will then mount the dental casts on an articulator using the wax bite to relate the casts to each other. Centric occlusion occurs when after the teeth make contact and then slide into a position where the teeth make the most contact. During the movement from centric relation to centric occlusion, the condyles change position. It must be noted that this is an important clinical and forensic distinction. As the jaws open the lower anterior teeth define an arc which is not a circle because there is rotation and translation. So a fixed axis of rotation articulator does not give the arc of closure and the change in position of the condyles and subsequently the teeth. There can also be protrusion of the lower jaw in opening and biting. Can a bite mark examiner determine the amount of protrusion, if any, on a bite mark?

Dental articulators at present are not capable of recording the rotation and translation in real time during the opening process because of the mechanical limitations. The technique involved in getting an articulator to perform in real time will be shown. Presently, dental casts of the maxilla and mandible are mounted in centric occlusion in the jaws of pliers to simulate the masticatory apparatus of the suspect. The examiners hands are used to supply the force in making a bite into a human, animal, or other material. As presented in the 2008 AAFS meeting in Washington, DC, centric occlusion is not the relation to use because the teeth during a bite never completely touch, if at all, so centric relation is the proper mounting, with the proper vertical dimension.

Additionally since machines have been developed that measure human bite forces, the forensic dental articulator can now generate a bite mark that is physiologically and anatomically correct.

The ability to capture the relationship between the jaws allows an identifiable bite mark pattern of anterior teeth, when only a few are present. Drag marks can be demonstrated and produced.

**Forensic Dental Articulator, Bite Mark, Arc of Closure**

## F20     Three Bites and You Are Out

*Richard R. Souviron, DDS\*, Miami-Dade County, Medical Examiner's Office, Number One on Bob Hope Road, Miami, FL 33136*

After attending this presentation, attendees will learn how trauma can and will occur to a body after the fatal event. It may occur by the first responders, at the hospital, in transportation to the morgue, or in the morgue. Further, second and third opinions are not always valid.

This presentation will impact the forensic science community by demonstrating how opinions can go astray when the circumstances of the event and history are unknown to the odontologist. The problem is compounded when a second or even a third expert agrees with the errant opinion. If all the experts have all the facts and the evidence is scientifically sound, a correct opinion should be independently produced by all.

A differential diagnosis of a human bite mark requires more than a photograph of the injury pattern in the morgue. The odontologist should know the circumstances of the event. This should include the history, the time from the event to the time the photographs were taken, and all scene photographs of the victim. The scene photographs are important to document body position, clothing, jewelry, and any object that may produce a "pseudo" bite mark. In cases where first responders find the victim alive, scene photographs of the victim are not going to be taken. Photographs of the victim in the E.R. or recovery room are part of the whole picture that the odontologist needs to determine whether the injury pattern is a human bite mark or not. Cognitive thinking dictates that all pertinent information be known before an accurate diagnosis can be made. Common sense also plays a part in the odontologist's evaluation of a pattern injury to determine if it is a human bite and the proper orientation. There have been mistakes in diagnosis of a pattern injury as a human bite mark and then in the analysis and comparison to a suspect. The systematic and scientific evaluation using common sense and proper cognitive thinking will reduce or prevent mistakes. In several cases where mistakes have occurred cognitive thinking was replaced with emotional, irrational and unreasonable thought process.

**Cognitive Thinking, Bite Mark, Odontologist**

## F21     Bite Marks, Bullets, and a Homicide in Reno

*Norman D. Sperber, DDS\*, 6237 Caminito Telmo, San Diego, CA 92111*

After attending this presentation, attendees will be able to recognize good bite mark patterns. This presentation led to the conviction of an individual – minutes before the expert was to testify.

This presentation will impact the forensic community by illustrating the steps involved in courtroom preparation. Although the maxillary arch was not as easy to visualize as the mandibular arch, the similarity in both mandibular bite marks was striking. The defense attorney was shown the three exhibits just before the expert testified.

On September 2, 2006, the Reno (Nevada) Police Department responded to a call at a single-family residence. At 6:15 a.m., a medical aid limited search of the home by investigators revealed a victim inside her residence lying on the floor next to a bed, fully clothed. The residence entry area, living room, kitchen, and master bedroom, where the victim lay, were strewn with broken and bloody items, which included clothing, lampshade, table, and a drinking glass. A .357 magnum revolver with expended casings inside was found on the hallway floor adjoining the master bedroom. Moments before, a defendant left the residence peacefully into Reno Police Department custody, following an hour-long negotiation. Prior to these events, the victim was last seen in Carson City alone at 2:00 a.m. The suspect, a musician, was last seen in a dance hall, alone, at 1:30 a.m. The victim, a professional singer, performed in Reno, Lake Tahoe, and the San Francisco Bay area. Both the victim and the suspect shared the four-bedroom home. The defendant was known to have a video camera while living at the victim's residence. His video camera was found positioned on a couch arm facing the master bedroom about five feet away with a blue sheer cloth hanging from the ceiling. The camera view screen was

activated and on when discovered. A household-gauge electrical extension cord extended from an adjoining bedroom about eight feet from the video camera. A notebook journal on the kitchen table had handwritten entries, an excerpt which reads, "There are things you want, including sexually, that I am flat out not willing to give at this time and I'm uncomfortable living in a situation with that hanging over me." Next to his journal was a note written by the defendant reading "I'm Sorry." The other journal entry excerpts read, "Life is more difficult with you living here." "I'm lying here shaking again. You're upset. I'm upset. This is not a good situation. At some point in almost every day, I'm paralyzed-afraid to do what I need to do for fear of upsetting you or upsetting the balance", and "If we'd spent ½ the time rehearsing that we do fighting, we'd have a night's worth of music."

The victim's autopsy revealed three gunshot wounds to her skull, multiple bruises, and abrasions on her face, torso, and extremities. Photographs of these injuries were provided by the Washoe County Sheriff's Forensic Science Division. The photographs revealed most of the above noted injuries including two human bite marks behind the right shoulder and back. In addition, there was an injury to one breast, which authorities believed could have been a bite mark. It was concluded that the mark on the breast was not a bite mark, so the investigation was limited to the two bite marks on the posterior portion of the victim's body.

The following report was forwarded to Washoe County Deputy District Attorney Bruce Hahn regarding the bite marks: "I have received a number of color photographs as well as a CD from the Reno Police Department and the Washoe County Sheriff of the victim in this case. Specifically, I was asked to determine if bruised lesions on the left breast, right shoulder, and back of the victim were human bites or not. I also received dental models of Mr. Pullin. I do not believe that the breast injury is a human bite. There are no arches (lineup of the teeth) or anything that resembles the incisal edges of the anterior teeth. Therefore, I will discuss the lesions found on the right back and right shoulder.

**Right Shoulder:** This lesion is a human bite mark and it reveals six maxillary (upper) teeth and seven mandibular (lower) teeth.

This bite itself is not ideal, because many of the marks are distorted and do not show the details of some teeth. The suspect has a very unusual alignment of especially the lower teeth. Although the tooth marks in the skin are fuzzy, it is still possible to visualize the lower teeth alignment. The same is true of the upper teeth. In this case, the unusual position of his upper teeth can be seen in the photographs. Therefore, it is highly probable that Mr. Pullin's teeth caused this bite.

**Right Back**: In this bite the same discussion applies to the quality of the bite. The lower teeth especially can be visualized in the bite mark. The upper teeth are not visualized as well as the shoulder bite. In fact, the central incisors hardly mark at all on the skin and the right side of the bite mark has three or four teeth that are conjoined. It is not unusual for the lower teeth to mark much better than the upper teeth. Thus, although it is difficult to visualize the upper teeth in this right back area, this fact is more than offset by the obvious relation of the lower teeth to the skin. Therefore, it is highly probable that the suspect is responsible for this bite mark as well. The rating system I use is as follows: (1) inconsistent, (2) consistent, (3) probable, (4) reasonable dental certainty. If the bite marks were more distinct, I would have used the term reasonable dental certainty. However, I have only used that term three times, due to the fact that the skin is not an ideal impression medium." The comparisons were made using tracings both electronically produced and by various hand-drawn methods. These tracings were almost identical. This presentation will demonstrate the photographic images and the unusual alignment of the suspect's lower anterior (front) teeth.

Just before the odontologist was about to testify in the afternoon, the "defendant simply plead straight up to the charge on file," said the Deputy District Attorney. Pullin, the defendant, faces a maximum sentence of life without parole, and another life sentence for the use of a deadly weapon.

**Bite Marks, Pleading Out, Preparation**

## F22 Simulated Intraoral Digital and Film Exposures (103) Using a Portable Hand-Held Dental Radiation Emitting Device to Determine Background (Scatter) Radiation "Safe Zones" for Ancillary Personnel Working in an Open Bay Environment

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After attending this presentation, attendees will understand the importance of maintaining ALARA principles when using a hand held radiation emitting device. The learner will be aware of the distances required to maintain a safe zone to avoid scatter radiation exposure to ancillary personnel from a hand held radiation emitting device.

This presentation will impact the forensic community by helping clinical and forensic dental personnel assess the safe distances required to reduce or avoid background (scatter) radiation exposure when a portable, self-contained, cordless, hand-held dental radiation emitting device is in use.

Exposure of ancillary personnel to background (scatter) radiation while using a portable, self-contained, cordless, hand-held dental radiation emitting device was determined while the operator employed typical use scenarios during the exposure of 103 simulated digital and/or film based dental radiographs at 0.25 seconds and 0.65 seconds respectively. The study was conducted in an open bay dental clinic environment, similar to what may be experienced in a morgue setting in which ancillary personnel may inadvertently pass through an area in which a radiation emitting device is in use. Background (scatter) radiation was measured with three ion chamber detectors positioned at three, six, and nine foot radii from the simulated oral cavity image plane. Results of the exposures recorded at these locations were compared with accepted, natural annual background scatter radiation exposure levels (360mR / year.) The ion chamber located opposite the radiation emitting source (Position No. 3) consistently received the highest readings. Extrapolation and comparison of this data to annual background scatter radiation levels of 360mR/ year indicated that an individual standing at position No. 3 would have to be exposed to1260 procedures per year in that position to receive 12.5% of the annual dose at 3 feet, 2.1% of the annual dose at 6 feet, and 1.9% of the annual dose at 9 feet. Radiation safety regulatory guidelines dictate that a six foot safe zone distance be maintained to follow the principles of ALARA for occupational workers when exposing dental radiographs. These findings will help clinical and

forensic dental personnel assess the safe distances required to reduce or avoid background (scatter) radiation exposure when a portable, self-contained, cordless, hand-held dental radiation emitting device is in use.

**Forensic Science, Radiation Safety, Portable Radiation Emitting Device**

## F23 Root Morphology and Anatomical Patterns in Forensic Dental Identification: A Comparison of Computer-Aided Identification With Traditional Forensic Dental Identification

*Dirk van der Meer, DMD\*, Box 208, 3300D Smith Drive, Armstrong, BC V0E 1B0, CANADA; Paula C. Brumit, DDS, 103 East Beltline, Suite H, Cedar Hill, TX 75104; Bruce A. Schrader, DDS, 9004 Francia Trail, Austin, TX 78748; and David R. Senn, DDS, 18 Villa Jardin, San Antonio, TX 78230-2749*

After attending this presentation, attendees will have gained further insight into the reliability of dental radiographs for identification purposes when restorations and coronal structures are not present. The dependability of root morphology, trabecular patterns, sinus outlines, and other anatomical features for forensic identification will be discussed. The effectiveness and threshold limit of digital subtraction software for determining similarity between antemortem and postmortem dental radiographs is evaluated. The error rates for the digital evaluations are compared with the error rates of forensic odontologists comparing the same radiograph pairs by traditional visual means.

This presentation will impact the forensic community by both discussing the reliability of forensic dental identification when limited antemortem or postmortem radiographic information is available and by providing insight into the usefulness of a computer program to aid forensic dental identification.

Because of the individuality of dental patterns, the resiliency of dental structures to withstand extreme conditions, and the accessibility of antemortem dental records, forensic dental identification plays an important role in establishing the identity of unknown decedents. Dental comparisons have played vital roles in victim identification following multiple fatality incidents such as the September 2001 terror attacks, the 2004 Indian Ocean tsunami, and the 2005 Hurricanes Katrina and Rita. Forensic dental identification can be an important identification method in smaller scale cases which involve single or multiple fatalities including motor vehicle crashes, smaller scale airplane crashes, structural fires, and whenever decomposed or skeletonized bodies are found.

Dental identification is a reliable and efficient method but often relies on the uniqueness of individual features of dental restorations compared between antemortem and postmortem radiographs. Identification is more challenging in cases in which either no antemortem dental restorations exist or no postmortem restorations remain after events resulting in fragmentation or prolonged extreme heat exposure. Root morphology, bone trabecular patterns, sinus morphology, or other distinctive characteristics are the primary anatomical features used for comparisons in these cases. However, the error rate for forensic odontologists performing visual identifications in these cases has not been quantified. Studies by Clement, Dove,

Anderson and others exploring the use of digital subtraction radiography to aid comparison of dental radiographs have rendered favorable results. Lehmann determined that cross covariance coefficient (CCC) was an appropriate statistical tool when used with digital subtraction radiographic comparisons. Flint et al used digital subtraction radiography to determine that there was a significant difference in CCC between images taken at different times from the same individual and those from different individuals. A web based study by Sweet and Pretty evaluated dental identification error rates and determined that comparing digital radiographs via the internet was a valid, accurate and reliable method. Clinical trials using actual forensic cases to test the usefulness of subtraction radiography and the error rates for traditional visual identification by forensic odontologists are needed. This is especially true for those cases in which there are no restorations and when coronal structures are missing postmortem and therefore not present for comparison.

Coronal structures on antemortem and postmortem dental radiographs from actual forensic identification cases were digitally removed using Adobe Photoshop (version CS3). Each case was represented by one antemortem and one postmortem radiograph. Analysis of the radiograph pairs was performed on a Toshiba Satellite notebook computer, using the Windows 98 operating system within Microsoft Virtual PC on Vista Premium operating system. Software used was UTHSCSA ImageTool Version 3.0 (developed at the University of Texas Health Science Center at San Antonio, Texas). Using the UT-ID plug-in module for ImageTool, each pair of AM-PM radiographs was registered to adjust for varying projection geometries. Subtraction radiography and pixel by pixel image comparison techniques were applied to determine the cross covariance coefficient.

A web-based participant examination was designed (using HostedTest[TM], Irvine CA). Participating forensic odontologists visually examined the same unregistered AM-PM radiograph pairs and established one of the four American Board of Forensic Odontology identification conclusions: positive identification, possible identification, exclusion or insufficient evidence. The same images were analyzed using UT-ID/ImageTool. Error rates for the forensic odontologists' visual identification were established and compared with the error rates using the UT-ID/ImageTool computer-aided identification method.

**Identification, Dental, Computer-Aided**

## F24 Postmortem Assaults on a Variety of Denture Labelling Systems

*Ray Richmond, MPhil\*, and Iain A. Pretty, DDS, PhD, Dental Health Unit, 3A Skelton House, Manchester Science Park, Manchester, Lloyd Street North, M15 65H, UNITED KINGDOM*

After attending this presentation attendees will appreciate the physical characteristics of a range of denture labeling systems and their resistance to extremes of temperature and pH.

This presentation will impact the forensic science community by highlighting the need for resilient, effective, and patient acceptable means of labeling dental prostheses.

The value of natural teeth (and associated oral tissues) to forensic dentistry is evidenced many times over in the dental literature. The edentulous patient on the other hand presents a more perplexing problem

as far as determination of identity is concerned. Whilst 16 -18 matching elements are usually required for a positive identification by fingerprint analysis, an appropriate number of comparison features for dental identification has not yet been established, owing to the infinite number of possibilities. Hence, in the case of the edentulous individual, the marking of dental prostheses provides an opportunity to give the anonymous/stereotype denture the uniqueness inherent to the natural dentition.

Forensic organizations worldwide have recommended that dental prostheses be labeled with at least the patient's name and preferably with further unique identifiers such as social security number etc. The practice of denture marking has been conducted over many years and several denture marking systems have been reported in the dental literature. However, very little is known about the resilience of such systems to conditions experienced in the majority of post- and perimortem assaults.

The purpose of this investigation therefore, was to expose a selection of denture labels (made from either paper, plastic, or metal) to a series of hostile environments, simulating conditions in which bodies may be found. One label included in the study was an RFID system consisting of an electronic data carrier, generally known as a tag or transponder. The tag consists of a torpedo shaped microchip with a coiled antenna, measuring enclosed within an 8.5mm x 2.2mm glass capsule.

The specimens were mounted into a 10 x 10 x 0.5cm block manufactured from Poly methyl methacrylate acrylic (PMMA) pink/veined denture base resin. One example each of the denture identification labels were then placed into the block to a depth of 2mm before being covered by clear self cure PMMA.

Postmortem assault conditions included:

- Burial of up to six months in acid soils of various levels of pH,
- Emersion in sea water for periods of up to 6 months,
- Emersion in fresh water for periods of up to 6 months,
- Emersion of up to 6 months in concentrated Milton disinfectant,
- Freezing in temperatures of approximately $-20^0$C for up to six months.
- Emersion in concentrated sulphuric acid for a period of 24 hours.
- Emersion in concentrated sodium hydroxide Na OH for 24 hours.
- Emersion in liquid nitrogen for a period of 3 minutes.
- Exposure to a naked flame until the specimen block caught fire and was allowed to burn.

Results of the study indicate that the majority of the denture labeling systems appear capable of withstanding a range common, and not so common postmortem assaults. With regard to thermal insult however, most performed badly with the exception of a label constructed from stainless steel orthodontic band. However, the RFID-tag performed above expectations in the majority of experiments. Furthermore, its cosmetic appearance has proven most popular with many patients.

**Human Identification, Postmortem Assaults, Dentures**

## F25 Image Quality Assessment of Two Portable Handheld Dental X-Ray Units for Forensic Odontologic Applications

*Patrick W. Thevissen, DDS\*, Pisha Pittayapat, DDS, Reinhilde Jacobs, PhD, and Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven, 3000, BELGIUM*

After attending this presentation attendees will be familiar with the evaluation of radiographic image quality acquired on two different image media being exposed by two portable x-ray devices.

This presentation will impact the forensic community by providing knowledge of the quality of dental x-ray images acquired with a portable AnyRay® and Nomad® unit in combination with CMOS and phosphor plates. The image quality will be assessed comparing exposures obtained with the fixed x-ray MinRay® device.

Dental radiography plays a major role in identifying unknown human body remains. Comparing antemortem and postmortem dental radiographs often results in establishing positive identification. Recently developed portable dental x-ray units increase the mobility of the forensic odontologist. They allow more efficient ad hoc x-ray work in a disaster field, especially enabling direct digital management and potential immediate matching when combined with a CCD/CMOS carrier.

In this study in vitro dental x-ray images exposed by two portable and one gold standard x-ray unit and captured on CMOS and phosphor plates are evaluated on image quality. In total thirty samples containing sound, decayed and restored extracted teeth, together with two exemplars of teeth containing skeletal jaw formalin-fixed teeth including the mandible were mounted with a radiolucent polyurethane spray foam on blocks and plates. This allowed standardized and parallel repositioning. A small silver cone includes the foam and serves as a reference point for further measurement. X-ray images are obtained with the MinRay® 60 kVp 0.14-22.4 mAs as gold standard x-ray unit and likewise with two portable x-ray units the AnyRay® 60 kVp, 0.02-4.00 mAs and the Nomad ® 60 kVp, 0.023-2.277 mAs on Durr® Dental phosphor plates and a Sigma® CMOS image medium. The effect of object-image receptor distance was checked for a mutual length of 0.8 and 2.5 cm keeping the object- source distance constant at 20cm. For each x-ray device all the available exposure times were run from low to high in every different parameter setting and for each sample. The acquired images were randomly presented to four observers for standard image quality and forensic diagnostic parameter quality evaluation on a 4-point rating scale and statistical assessed. A pilot set up with phosphor plate images acquired from gold standard and AnyRay® unit linear measurement for forensic purposes were compared, and tested for inter- and intra-observer variability.

This study indicates the applicability of both portable x-ray units regarding the overall image quality for forensic diagnostic applications. In the pilot set-up significant differences in tooth length, pulp length and root width were found, with enlarged dimensional measures for the AnyRay module around 6%.

The feasibility of the tested portable hand-held dental radiation emitting devices for forensic odontologic identification and certain specific dental age estimation purposes is based on acceptable image quality results and sufficient accuracy for particular forensic measures.

**Human Identification, Portable Radiation Emitting Devices, X-Ray Image Quality**

## F26 A Useful Case Study for the Cleaning of Dentition for Forensic Odontological Analysis

*Lindsey Glock\*, and Nicole A. Wall, MFS, College of Saint Mary, Forensic Science Program, 7000 Mercy Road, Omaha, NE 68106; Kenneth P. Hermsen, DDS, Creighton University, School of Dentistry, 2500 California Plaza, Omaha, NE 68178; and Cheslee T. Cornell, College of Saint Mary, Forensic Science Program, 7000 Mercy Road, Omaha, NE 68106*

After attending this presentation, attendees will know the composition of the material that collects on the teeth after death related to decomposition and understand some of the difficulties in cleaning the teeth of decomposed victims for accurate examination. They will also be given specific recommendations, based on the results of the study, to clean the decomposition products from the teeth.

This presentation will impact the forensic community by providing forensic dentists practical information and products to quickly, safely, and efficiently clean the decomposition material from the teeth of the victims in a mass fatality incident to provide for accurate oral examination.

Decomposition products were collected from the teeth of three pigs and analyzed for chemical composition. Various available cleaning products were tested for their ability to quickly and efficiently remove the decomposition products from the teeth. Results indicated that the disinfecting wipes, containing N-alkyl, dimethyl benzyl ammonia chloride and dimethyl ethybenzyl ammonium chloride, proved to be most efficient in removing the decomposition products.

In a mass fatality incident, accuracy and efficiency are both essential elements for a successful operation. For forensic odontologists, removal of the products of decomposition from the teeth of the victims is a time-consuming but important function. Removal of the decomposed material is required for an accurate oral examination of the victims. As esthetic dental restorations become less identifiable clinically, the need for thorough removal of the decomposed products becomes even more important. In the past, several agents have been used to try to clean the material off the teeth. Among those that have been commonly used are alcohol, sodium hypochlorite, and hydrogen peroxide. Each of these have advantages, but all the commonly used cleaners leave behind a greasy layer of material. In addition, the products can be incompatible with one another. For example, hydrogen peroxide can't be used in conjunction with sodium hypochlorite, which is one of the materials that can be used for decontamination, due to a chemical reaction that causes formation of a significant amount of foam. An ideal cleaning material used to remove the products of decomposition should possess the following properties: commercially available, easily obtained and stored, non-toxic, efficient for the task, able to be used in unventilated areas, and be compatible with materials used to decontaminate the remains.

The purpose of the study was to examine the chemical makeup of decomposition and investigate various cleaning materials to determine which one(s) most closely meet the desired properties. Three pigs were sacrificed and allowed to decompose. The swine were varying aged piglets, somewhere in the range of 1-2 months in age. The first one weighed approximately 11.5 kg, measured approximately 80 cm, and was buried approximately 2 ft underground. The second weighed approximately 21.2 kg, measured approximately 92 cm, and was buried approximately 2ft underground and the third weighed approximately

15.6 kg, measured 86 cm, and was placed above ground, secured within a wire mesh screen cage.

When decomposition had progressed to the desired level, the jaws were removed and a portion of decomposed material was removed for chemical analysis. The teeth were then cleaned with various commonly available cleaning products to test their efficacy and efficiency. The cleaning materials were compared to isopropyl alcohol which commonly has been used to remove the decomposition material in the past. Three evaluators evaluated the cleaning efficiency (time and effort) and efficacy (cleanliness) of the various products and rated them against the alcohol control. Of the materials tested, the disinfecting wipes, which contains ammonium chloride, proved to be the best in removing the decomposed material. The disinfecting wipes met all the criteria for an effective cleaning material.

**Forensic Odontology, Decomposition, Mass Disaster**

## F27 Detection of Pit and Fissure Sealants Using UV LED Light

*Gerald Guzy, DDS\*, 259 Kinderkamack Road, Westwood, NJ 07675*

After attending this presentation, attendees will gain knowledge of the usefulness of UV LED light in detecting the presence of pit and fissure sealants during the forensic dental examination.

This presentation will impact the forensic science community by demonstrating that the use of small, inexpensive, battery operated UV LED lights can make the presence of pit and fissure sealants more easily detected by the examining forensic odontologist.

The development of pit and fissure sealants has been shown to be effective, efficient, and a safe method of preventing pit and fissure caries in children. The increasing use of sealants will help to prevent the caries process and reduce the need for invasive procedures such as amalgam and resin based composite placement to restore dental caries. Ultimately, this may prevent the need for more advanced procedures such as endodontic treatment, extractions, crown and bridge, and implant placement.

The caries free/restoration free individual is becoming more common requiring the forensic odontologist to use anatomical landmarks such as root size and shape and bone patterns for forensic dental identification. Many of the caries free/restoration free individuals have pit and fissure sealants which would be useful for forensic dental identification.

Pit and fissure sealant materials are available as clear, opaque, tooth colored, white, and tinted. However, sealants may be difficult to detect visually, radiographically, or with a dental explorer and may be overlooked during the forensic dental examination. Sealants applied to permanent molars in six to eight year old patients may be significantly worn and difficult to detect in a teenager or young adult.

UV light has been used in the evaluation of fluorescence of resin based composite restorations. The fluorescent properties of resin based composites when exposed to UV light has been studied and revealed that some composites fluoresced brighter or darker than the surrounding tooth. In addition, a small inexpensive battery operated UV LED light was used in determining the presence of resin based composites in a recent forensic dental identification of a severely decomposed body.

The purpose of this preliminary study was to evaluate the use of UV LED lights at 365nm and 395nm for the detection of pit and fissure sealants.

A total of eighteen extracted noncarious nonrestored human molars were used in this study. The occlusal surfaces were cleaned with a slurry of oil-free pumice and water, and the teeth were stored in distilled water until used. Sixteen different pit and fissure sealants from nine different manufacturers were applied to the occlusal surfaces of sixteen different teeth. Two teeth were left unsealed as controls. Eleven teeth had filled sealants applied, and five teeth had unfilled sealants applied. The sealants were cured with a Morita Jetlight 5000 LED curing light R. The light output was measured using the radiometer built into the charging base of the curing light. The light output was measured each time a sealant was cured and was consistently greater than 900 mW/cm2. Curing times were based on the sealant manufacturer's recommendations.

The teeth were then examined using standard overhead fluorescent lighting, then re-examined in a darkened room using a Nichia 365nm 5 LED UV light R and a Inova X5 395nm 5 LED UV light R. These lights were chosen because they are small, inexpensive, easily obtained, and battery operated.

In general, pit and fissure sealants appear darker than the surrounding tooth structure when illuminated with UV LED light due to the absorption characteristics of the sealants as compared to the fluorescence of the natural tooth. UV LED lights at 365 nm and 395nm both enhance the appearance of pit and fissure sealants. The presence of pit and fissure sealants was easier to detect using the 365 nm UV LED light as compared to the 395nm UV LED light. The sealants appeared darker than the surrounding tooth with the 365 nm light than with the 395 nm light.

The results of this study suggest that the use of small, battery operated UV LED lights can be valuable in the detection of pit and fissure sealants during forensic dental identifications; however, their use does not preclude a thorough visual and radiographic examination.

**UV LED Lights, Pit and Fissure Sealants, Forensic Odontology**

## F28 The "Transformation" of a Simple Case of Identification Into a Complicated One

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After attending this presentation, attendees will see the importance of compiling and filing dental and medical records, including radiographs.

This presentation will impact the forensic community by demonstrating how a simple case of personal identification can become a hard case without complete dental and medical records.

Traditional methods used in forensic identification are based on the comparison of antemortem (AM) and postmortem (PM) radiological images, and they are often a valuable alternative to fingerprinting and DNA analysis. In addition, radiological images are only of value in making positive identifications in cases where there are AM images available for comparison.

Dental identification utilizes general teeth, jaw, and orofacial characteristics, as well as the specific features of dental work including metallic or composite fillings, crowns, bridges, and removable prostheses. Dental identification also takes into account the distinctive configuration of bony structures of the jaws (i.e., mandible and maxilla), the presence and shape of teeth (including the roots), the configuration of maxillary sinuses, and longstanding pathologies (such as prior fractures and orthopedic procedures).

In this case the official consultants from the Court of Lecce, Italy were asked to carry out all necessary procedures in order to establish whether the skeletal remains of a subject found in the countryside in 2002, could have been those of a man who had been missing since 1989. Anthropometric analyses established that the subject was a Caucasian male, aged between 23 and 31 years. Among the useful elements available, was a fixed circular prosthesis of very high quality and made of porcelain-alloy.

Other useful elements were significant fractures (humerus, tibia, and fibula), all of which had been mended using "surgical" screws.

Despite the presence of these elements, no antemortem radiographs were available from the hospital where the subject had been admitted, which could have positively identified the remains. Furthermore, when the subject's presumed dentist was contacted, he recognized the prosthesis as his own work, but no paper or radiographic documentation was available which could have provided absolute certainty as to the subject's identity.

In the end, because of the existence of a presumed brother of the deceased subject in question, DNA extraction from pulverized bone tissue was carried out for the purpose of comparing it to a sample of blood taken from the presumed brother.

Because the presumed brother and the remains of the subject were both male, investigation focused on the y chromosome. DNA typing of the DYS391, DYS389 I, DYS439, DYS389 II, DYS393, DYS390, DYS385, DYS438, DYS437, DYS19, DYS392 systems was carried out on the samples. Amplification was performed using the Thermal Cycler-DNA Gene Amp® PCR System 9700 (Applied Biosystems, Foster City, CA), and the AmpFℓSTR® Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA).

Analysis of the amplified allele fragments was performed by capillary electrophoresis. Identification of the genetic features of the samples, related to the DNA polymorphisms investigated, was performed by using an allelic ladder which included the major Caucasian variations. The results showed compatibility with nine out of the eleven systems, thus confirming the identification of the subject.

Positive identification of the subject in question was ultimately possible through DNA analysis. Notwithstanding the fact that other, very convincing features were present on the skeletal remains, which could have certainly made identification a much simpler task, the lack of antemortem radiographic images did not allow for positive identification. This case, in which many years had passed from the time of the subject's disappearance to the discovery of his skeletal remains, stresses the importance of compiling and filing dental and medical records, including radiographs. No regulations which require private dental practices to maintain dental charts of patients currently exist in Italy. As a result, dental records are often non-existent and when they do exist, their quality is often very poor and of little use.

**Personal Identification, Odontology, Case Report**

## F29 A Retrospective Analysis of the Forensic Files at One of the Dental Faculty Archive in Turkey

*Humeyra Kocaelli, PhD\*, Istanbul University, Istanbul, TURKEY; and Feryal Karaman, PhD, Istanbul University Institute of Forensic Sciences, Cerrahpasa, Istanbul, TURKEY*

After attending this presentation, attendees will see the importance of forensic odontology during the dental education.

This presentation will impact the forensic science community by showing that forensic science will take more importance in coming years.

In this study, 870 files have been searched at Istanbul University Dentistry Faculty which were saved at the Faculty Dean's archive between February 22, 1984 and June 12, 2008. Among these files, 249 trauma cases, 104 malpractices, 304 files with missing documents, and 213 of other subjects (such as expert report requests, arrested person's health check requests, price and check up investigation letters among the related departments, patient request and claims applications, etc) have been found. Traumas consisted mostly of maxillofacial traumas, traffic accidents, weapons, and other injuries. Most of the claims were concentrated in prosthetic treatments, where surgical and orthodontic treatments were followed in 104 malpractice cases.

During records review, it was realized that 285 of the 304 files with missing documents were associated with cases before 1990. Filing and properly retained documents were achieved with greater care after 1992. Distribution of documents according to the related official departments, revealed that requests and claims by the deans started from 1992 and incremental increase seen by 2003. This increment may be related with the ISO 2001 Certificate of Istanbul University Faculty of Dentistry that was taken in 2004. Before 1992, investigative affairs generally took place in police stations or by public prosecutors. As time passed, they were transferred to the courts and this has increased since year 2000. Fewer investigated malpractice cases were found in past years, but have also increased since 2000. The reasoning is that patients may understand their rights and also new laws regarding malpractice have been implemented or revised.

During investigating the reports with missing documents, it was discovered that the dentists did not have enough information and knowledge in how to avoid malpractice affairs. In addition, they did not have the knowledge for preparing a forensic report. Due to that reason, we are recommending Forensic Odontology lectures should added to the general forensic medicine lectures in dental curriculums.

**Forensic Odontology, Forensic Files, Retrospective Study**

## F30 Radiographic Images of Dental Implants as an Aid to Human Identification

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After attending this presentation, attendees will have an understanding of the importance of identifying dental implants for forensic purposes through their radiographic image with the aid of an archive of radiographic dental implants images.

This presentation will impact the forensic community by demonstrating how forensic dental radiology and human identification can benefit the recognition of dental implant manufactures found in forensic casework.

Dental prosthetic rehabilitation with fixed crown or partial/complete dentures supported by titanium implants is a very common treatment. There are a great number of implant systems of different designs available on to dentists, distributed on a national and/or international basis.

Forensic dental identification of an unknown decedent is a process which involves taking X-ray images of jaws in order to reveal as much information as possible about the deceased.

Forensic odontologists use, in fact, radiographic evidence to outline a profile of the unidentified remains. Likewise, clues gleaned from the type of implants used could also give direction, or narrow, the field of the investigation. Because dental implants from the various producers differ in shape and design, a variety of implant radiographic images must be expected. However, a catalogue of radiographic images of dental implants is not yet available.

The research began by collecting specimen implants from different manufactures with the aim of creating an archive of radiographic dental implant images. Fourteen dental implant manufactures replied sending multiple implants of various designs.

Digital radiographs were taken of all the implants donated at 0º, 30º, and 60º of horizontal rotation combined with -10º, 0º, and +10º vertical inclination relative to the radiographic beam and the x-ray sensor, in order to mimic clinical situations. A total of nine images per implant were taken and recorded in the archive.

The survey should be considered a work in progress, as the archive has still to be enlarged. A worldwide radiographic implant image database, including similar "cloned" implants, would be an enormous help to both forensic odontologists and prosthodontists in identifying pre-existing implants.

**Dental Implants, Dental Radiology, Human Identification**

## F31 The Effect of Different Dental Coding Methods on Victim Identification

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After attending this presentation, attendees will explore the effects of different dental coding methods when they are applied to the WinID program. This study compares the resultant ranks generated with detailed dental codes versus ranks generated with very simple dental codes.

This presentation will impact the forensic community by presenting evidence-based research on the effects of different dental coding methods with WinID. These results will be of interest to forensic odontologists working with mass fatality events and victim identification.

The goal of any computerized dental matching software is to rank potential matches between antemortem and postmortem dental data. These rankings greatly assist forensic odontologists in the victim identification process by providing them with a list of the most likely

matches. This study examines the effects of different dental coding strategies using WinID.

Dental case data were formatted in two manners: "detailed" and "simple." For the detailed format there were 34 possible codes for each tooth, including / (no Information), V (virgin), X (missing), and 31 possible combinations for surface restorations (MODFL). For the simple format there were only four possible codes per tooth / (no Information), V (virgin), X (missing), or F (filled/restored tooth). In the "detailed" system, all the surfaces with treatment were recorded. In the "simple" format, any restored tooth received a code of "F" regardless of the extent or location of the restoration on the tooth. A multi-surface restoration in the detailed format might be coded as "MODF," while in the simple format it would be coded "F" (note that F in the detailed format stands for "Facial" while in the simple format it stands for "filled/restored." Utilizing existing codes allowed different coding methods to be evaluated by the WinID ranking algorithms).

Two distinct samples of data were utilized. The first set was compiled from two large-scale dental health studies (NHANES and TSCOHS) and was used to test the "ideal" scenario of accurate, up-to-date, perfectly matched dental records. Although this dataset may not be a realistic representation of a true forensic scenario, it does provide a theoretical framework to observe the effect of coding strategies without interference of outside factors such as data entry or coding errors. The second dataset utilized the World Trade Center disaster dental data in order to see if the trends observed with the "ideal" data were also observed with real world "imperfect" data.

In order to simulate different disaster scenarios, the "ideal" data was further divided into different groups. These groups represented situations ranging from a small number of victims with relatively complete bodies to a large number of victims with body fragmentation. Fifty random records were selected as the *postmortem sample*. In order to simulate postmortem fragmentation, three scenarios were created for each "victim": (1) intact body (28 observations), (2) fragmentary body (14 observations), and (3) very fragmentary body (7 observations). *Antemortem samples* were constructed to simulate varying disaster population sizes of 50, 100, 500, 1000, and 10,000 individuals. This system allowed trends to be observed based on varying numbers of observable teeth and differing population sizes.

For the WTC test data, 50 postmortem cases were randomly selected that had 14 or more dental observations present. These records were compared against an antemortem database of 2,464 records. Any record that did not contain dental information was excluded.

Utilizing WinID, rankings were generated for the 50 postmortem cases using both the "detailed" and "simple" code formats. The number of records that were tied with or better than the correct match was noted for each case and each data format. Differences between the "detailed" rank and the "simple" rank represented the effects of the coding format (i.e., better, worse, or no change). Although this method produced higher ranking numbers than an "absolute record number" system it eliminates any bias introduced when multiple cases receive the same ranking in WinID. In addition, sample data showed that "absolute record number" ranks produced similar trends.

Results from the "ideal" data show that for smaller disasters with less fragmentation there is little benefit in utilizing detailed coding. As disaster size and fragmentation increase, ranking degradation occurs when codes are simplified. The results from the WTC data support this overall trend. Based on the large number of WTC victims, the "detailed" coding format generally outperformed the "simple" coding format.

Obviously computer ranking systems, such as WinID, are only tools to provide a starting point for forensic odontologists to make dental comparisons. Simplification of dental codes expedites the data collection process and, based on ideal test data, appears to have minimal impact on computer ranking in small to medium datasets and incidents with few fragmented specimens. The major shortcomings of the simple coding system are the loss of the ability to search for unusual restoration patterns and poor rankings with high fragmentation/large population scenarios. Future studies should explore whether the trends observed in this study are also seen with other case data and with other dental ranking programs.

**Dental Coding, Victim Identification, WinID**

# F32 Cracking Coding Dilemmas in Missing Persons Cases

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After attending this presentation, the attendees will understand how the forensic odontologist can assist law enforcement in the proper recording of the dental records of long-term missing persons.

This presentation will impact the forensic community by highlighting the importance of the forensic odontologist in missing persons cases.

This presentation will show how pertinent dental information entered by the forensic odontologist can assist investigators in solving missing person's cases. The comparison of a missing person's dental records with the dental evidence from unknown remains has long been recognized as one of the most reliable means of positive scientific identification. The services of a forensic odontologist rather than the family dentist should be utilized for recording missing person's dental records. The forensic odontologist is extensively trained to recognize the subtle nuances in dental chart interpretation that, if recorded erroneously, will cause a misidentification. The forensic odontologist will also be aware of those rarely occurring anomalies that warrant attention and publication to the appropriate agencies for securing victim identification.

As of July 2, 2008, in New Jersey, there were 1,349 missing persons entered into the National Crime Information Center (N.C.I.C.) database. Of this number, only 133 missing person's records include dental charting. In contrast, dental charting has been entered on most unidentified person's records from New Jersey into N.C.I.C., if teeth are recovered. This discrepancy can be attributed to the utilization of forensic odontologist by the New Jersey County Medical Examiners that do a forensic dental examination on the unidentified person. Because of the low number of missing person's records with dental, an initiative is underway at the Forensic Anthropology Laboratory, Office of Forensic Sciences, and New Jersey State Police, to use forensic odontologists to chart, radiograph, and code the dental records of all missing persons. A secure central repository for New Jersey long-term missing person's dental records has been established. This allows for accurate dental entries to be made into N.C.I.C. and other national missing person's databases.

A recent case involved the discovery of skeletonized remains in New York state, with a nearly complete dentition. All thirty-two teeth were fully erupted and present except for #23, #24, and #26 that appeared radiographically to have been lost through postmortem

avulsion. What was strikingly unique was the presence of two bony impacted teeth found distal to the mandibular third molars.

Recognizing the importance of this information, the New York forensic odontologist notified the New York investigating officer. After explaining the rarity of the decedent's dental condition, the odontologist suggested contacting the New York State dental licensing department or the New York State Dental Society to request their assistance by emailing the description of the dental information to their list of dentists. A New Jersey forensic odontologist, who remembered recently entering a New Jersey missing person with similar dental charting into the New Jersey centralized dental database, notified the New Jersey State Police Forensic Anthropologist and the New York investigating officer. A tentative identification was made by examining an emailed photograph of the decedent's panoramic x-ray. The local New Jersey law enforcement agency was notified and the identification was confirmed after the original dental film was brought to the Medical Examiner's Office and compared by their forensic odontologist.

Utilizing trained forensic odontologists in missing person's cases will allow maximum potential for positive identifications when compared with unidentified remains.

**Forensic Odontology, N.C.I.C., Missing Persons**

## F33 Blame Canada: Making Sense of Cross-Border Missing Persons/Found Human Remains Comparison Algorithms and Data Entry Forms

*Robert E. Wood, PhD\*, Princess Margaret Hospital, 610 University Avenue, Toronto, ON M5G 2M9, CANADA*

After attending this presentation, attendees will become aware of the problems and the proposed solutions when using antemortem and postmortem dental data coded on Canadian forms as well as efforts to create a system where the U.S. missing person's forms and the Canadian missing person's forms and their corresponding postmortem forms can effectively communicate with one another.

This presentation will impact the forensic community by making people in North America aware of the profound differences in methods used to code, input data, and make comparisons in National coding databases. Further it will emphasize the importance of completing postmortem and antemortem forms and images in a fashion that can be coded with CJIS and the dental image repository respectively.

There are thousands of unidentified bodies in North America and many more reported missing persons. There have been several organizational attempts to match dental records of the missing persons to those of found human remains. The problem is compounded by the relatively porous border between the United States and Mexico and the free movement across the Canadian / U.S. border.

All dental search programs depend on search algorithms that in turn depend on reasonably accurate entry of antemortem dental information from the charts of missing persons onto standardized data collection forms and similar information coded onto postmortem data collection forms. Accuracy is lost when information is coded by dentists that are not trained to do so.

Currently, both antemortem and postmortem dental coding in Canada are not uniform. Some geographic locales use a form that codes only tooth present, tooth absent, tooth restored. Other geographic locales use a form similar in part to the present U.S. NCIC dental coding forms. In the latter, tooth surfaces restored are coded – in the former tooth surfaces are not coded. Even within Canada there are areas where antemortem coding uses surfaces of teeth as primary data points and postmortem coding where tooth surface information is not used – and vice versa. The problem is compounded by the use of the universal tooth-numbering system in the United States and the federation dentaire internationale F.D.I. in Canada and elsewhere. A solution is proposed wherein one antemortem dental coding form and another postmortem dental coding form that account for differences in dental nomenclature may be used in Canada. This form is optically and informationally similar to the existing NCIC forms and can be placed without modification on the U.S. dental database. It also gathers all data required by both jurisdictions. Examples of the forms will be provided and sample data from the Province of Ontario's existing found human remains database will be presented. This last item could easily mesh with the NCIC dental image repository.

**Forensic Odontology, Unidentified Remains, Missing Persons**

## F34 Applications of the Implementation of a Soft Tissue Thickness Data Base Into a Flexible Statistical Model of Face Shape for Computerized Forensic Craniofacial Reconstruction Purposes

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The goal of this presentation is to demonstrate a computerized semi-automatic 3-D forensic cranio-facial reconstruction tool. This forensic application is based on a large scale database of facial soft tissue depths of Caucasian adults and a flexible statistical model of face shape used in computerized three-dimensional (3-D) craniofacial approximations.

This presentation will impact the forensic community by presenting a large scale database of facial soft tissue depths into a 3-D forensic cranio-facial reconstruction tool which allows for specific correction of gender, age, and body posture.

Mass communication of forensic facial reconstruction models in unsolved identification cases can stimulate recognition by relatives and may provide records to accomplish further comparative analysis. The majority of the reconstruction techniques use earlier published facial soft tissue depth charts collected on cadavers or in vivo. Traditional 3D facial reconstruction techniques apply modeling clay or Play-Doh® on a cast of the skull, approximating the estimated tissue depths at the landmarks, and interpolating in between. Recent different computerized techniques are evolved to obtain more objective 3D facial soft tissue estimations. In this presentation the application of the implementation of soft tissue thickness described by De Greef et al (2006) into a flexible statistical model of face shape developed by Claes et al (2006) is demonstrated.

De Greef et al performed in vivo facial soft tissue depth measurements on 967 adult Caucasoids employing a user-friendly, fast, mobile, and well validated ultrasound measuring device. Data of both

sexes, varying in age and body mass index (BMI) were collected at 52 manually indicated facial landmarks. The "A-scan" industrial ultrasound device was selected to perform the tissue dept measurements because of its low weight, compactness, facile transport and its ability to connect a 6mm diameter, 10MHz ultrasound transducer which can easily be pointed to the landmarks during analysis. The repeatability of the ultrasound measurements was tested on a subset of 33 volunteers and their accuracy proved after comparing the ultrasound measurements and the soft tissue thickness calculated from total head CT-scans on twelve patients.

The computer-based combined flexible statistical model for craniofacial reconstruction established by Claes et al requires the achievement of a skin surface and tissue depths database, a statistical face and soft-tissue depth model and a statistical model fitting procedure. The skin surface shape of approximately 350 individuals were captured with a mobile 3D photographic device, after measuring thickness and marking the 52 soft tissue landmarks and registering age, gender, and BMI of all the individuals. The constructed statistical facial surface and soft tissue depth tissue model consists of a geometrically averaged facial template together with a correlation-ranked set of modes of principal variations or face-specific deformations that capture the major changes or differences between facial outlooks and their skull-based landmarks in the database. The created elastic mask is subsequently fitted to the external surface of the individual craniofacial skeleton such that all the 52 landmarks of the mask fit the corresponding target skull-landmarks and the estimate of the nose tip.

Multiple reconstructions of the same skull but with different combinations of age, gender and BMI can be made within a few seconds. More specific facial soft tissue changes during aging can be simulated. The automatic adjustment or improvement of the model using face specific modes of variation, results in unbiased and more realistic 3D facial soft tissue reconstructions.

**Human Identification, Facial Soft Tissue Data Base, Forensic Facial Reconstruction**

## F35    Hidden in Plain Sight and Who's Your Daddy:  Cases of Interest to the Forensic Odontologist

*Allan A. Raden, DMD*, 814 North Delsea Drive, Glassboro, NJ 08028*

After attending this presentation, attendees will be familiarized with some unusual death circumstances requiring the services of the forensic odontologist. Some useful tips for securing antemortem dental records will also be provided.

This presentation will impact the forensic and dental community at large as an example of record keeping that could challenge an investigation. The use of DNA analysis will also be exemplified.

Occasionally, investigators have only half of the information needed to complete an investigation. For example, an individual claims to have committed a murder and hidden the body of his victim. Police may charge the individual for his actions, but until the victim's body is recovered and scientifically identified, it may not be proved that a crime has actually occurred. It could be months or years until all the pieces of a case are brought together for proper resolution.

Another example could be a missing person where there is no evidence of foul play. It is difficult to investigate a person who may choose not to be found or may be well concealed by circumstances of death.

The two cases described in this presentation demonstrate the role of the odontologist in the identification of persons who have died in unusual circumstances.

The first case involves a missing young man who either fell or was pushed to his death and remained undetected for some time even though a thorough search of his neighborhood revealed nothing. The ultimate discovery of his remains was not by investigators, but rather someone looking for something else. He was found in a most unusual location, almost a year after his disappearance. This individual was listed with NCIC and featured on *America's Most Wanted*.

The second case involves an alleged crime of murder linked to a family dispute. An abandoned live baby was the first clue to the disappearance of a young woman, yet no remains were discovered until months later even after exhaustive searches of the area where investigators suspected the remains to be. Even though a family member claimed responsibility for the missing person's disappearance, no evidence of murder was found. A very bizarre family situation had emerged from the investigation of this untimely death. This case is particularly interesting because of the difficulty in obtaining antemortem dental records in spite of postmortem evidence of extensive dental treatment. The delay in securing adequate antemortem dental radiographs compelled investigators to employ alternate methods of scientific identification. The difficulties with the antemortem dental record search and the unusual source of records associated with this case will be discussed.

**Dental Records, Dental Identification, Digital Radiography**

## F36    The Identification of The Victims of Flight 5191:  Keeping It Simple

*Mark L. Bernstein, DDS*, Department of Diagnostic Sciences, University of Louisville, School of Dentistry, Health Sciences Center, Louisville, KY 40292; and Corky Deaton, DMD*, 359-C South 4th Street, Danville, KY 40422*

After attending this session, attendees will see the benefits of using simple techniques along with a focused and organized approach to victim identification in a mass disaster.

This presentation will impact the forensic community by demonstrating the advantages of simplifying the odontology process in order to expedite identification of mass disaster victims.

On the morning of August 27, 2006, Comair Flight 5191 crashed on takeoff from Lexington, Kentucky bound for Atlanta, Georgia, killing 49 of 50 individuals on board. Only the co-pilot survived. The Chief Medical Examiner of Kentucky activated the mass fatality team of coroners, pathologists, anthropologists, and dentists.

Five local forensic dentists and a dental hygienist assembled at the Medical Examiner's facility in Frankfort, Kentucky, which served as the temporary morgue. The Commonwealth of Kentucky has, as do most jurisdictions, a mass fatality protocol that served as a template for action. Each mass disaster presents unique challenges that necessitate modifications in the management of the identification effort.

The identification process was conducted in the following manner:
The labeled, partly charred bodies were autopsied, then in assembly line manner, delivered to the dental postmortem section. Prosectors removed jaws while the dental hygienist served as a clean-handed scribe, taking notes regarding the status of each body and recording findings noted during jaw resection. Resected jaws were placed in labeled bags and transferred to two dentists for

photography and charting, following which the jaws were returned to the body bag. A coroner accompanied each body at all times so that no evidence was displaced. Meanwhile, the morgue personnel used the flight manifest to contact victims' families and treating dentists for antemortem records. Since most victims were local residents, area dentists responded rapidly and compassionately. Antemortem records were charted on a form similar to the post-mortem form.

The comparison section by and large did not rely on computers or radiographs. Victims' dentitions were intact. All victims were adults and most had significant dental restorations. Antemortem and postmortem records were divided into males and females (about 20 of each); the remainder were those on whom gender was not determinable. The most characteristic one or two findings in a given postmortem record were targeted and searched for among the antemortem records. Typically, this could be matched in less than three minutes, allowing a tentative identification. The residue of victims that had no characteristic match points because of inadequate antemortem records or non-characteristic findings were identified by making postmortem digital radiographs to correspond with antemortem films.

Dental identifications were made on 47 individuals, most within three days. Two individuals were identified on the basis of medical findings and exclusion of all others. Oral findings at the time of resection were also important in assisting the Medical Examiner with determination of cause of death (fire, smoke inhalation or blunt force injury).

This presentation will focus on the factors that allowed for expedient identifications of the decedents:
- Preplanning and organization,
- Communication between and among sections,
- Size and nature of facility,
- Equipment,
- The concept of "Keeping it simple and focused."

**Simple, Organization, Mass Disaster**


## F37 The Norwegian ID-Commission in Thailand After the Tsunami and a Critical View on the Dental Examinations and Comparisons

*Tore T. Solheim\*, University of Oslo, Box 1109 Blindern, 0317 Oslo, 0317, NORWAY*

After attending this presentation, attendees will acquire knowledge about the Norwegian ID-commission and the principles used in the dental registrations and comparisons. Also an understanding of the basis for criticism of the computer reports and the difficulties of international operations will be obtained.

This presentation will impact the forensic community by giving a better understanding of how to make ante- and postmortem dental reports and comparison reports on Interpol forms. Also a possible quality improvement in the use of these forms as well as other types of forms may be the goal.

The official Norwegian ID-commission consists of police, forensic pathologists and forensic odontologists and is intended for identification both in Norway and abroad when Norwegian citizens have perished. The question of identity is resolved only when all three professions agree and they should all sign the final ID-report. A total of 81 Norwegian citizens were missing after the tsunami in Thailand on December 26, 2005. All were found and identified. Of these, 77 were examined and identified by the identification team. The Norwegian team was sent to Thailand on December 29th, joined the international team, and participated in examination of all bodies including the transcription of dental records to the computer program DVI System International from Plass Data. The team also took part in the comparisons or reconciliation as it was called in Thailand.

Dental records for missing Norwegian citizens were collected at the Central Criminal Police Bureau in Oslo, transcribed into the computer program, and all records and radiographs were digitally photographed. The registrations and photos were transferred electronically to Thailand, while the original material was kept in Oslo. This is the preferred procedure; however, most countries sent the original material directly to Thailand without translation into English and with the risk of loss. The official reports from Thailand on the AM forms showed that dental information from dentists were recorded in 64 cases, of which 11 had only radiographs. A number of the missing Norwegians were young children. Only 3 records had no radiographs, but in the majority of cases (36) the radiographs were only bite-wings which may be suboptimal for the identification process.

Postmortem registrations were made on printed forms and later transferred to the DVI computer program. As one never knew who was Norwegian or not, only a few were examined by Norwegian teams. The remaining was examined by different teams of other nationalities. Official dental reports from 76 individuals showed that in only 59 (77%) cases was the name of the examining dentist indicated. Even worse was that in an unknown number of cases, the dentist who transcribed the information into the computer program was given as examining dentist. The Interpol form F1 has a field where the condition of the body, including head, teeth, and eventual injuries should be described. Only in three cases was this field filled in and only by Norwegian dentists. There was great variation in how the field was filled in or completed. When many dentists describe sound teeth as teeth present it is imprecise. Field 91 in form sheet F2 was filled in only in 23 (44%) cases out of 52 which could be examined. Often the only indication was child, young adult, etc. Only Norwegian dentists had indicated age (e.g., approximately 20 years). Often these indications were almost exactly correct. Dentists are extremely good at judging the age based on teeth; however, this ability is not often utilized. In Norway, the age must be assessed visually in all cases of dental identification.

The efficiency in the reconciliation could have been greatly improved by better organization. For the missing Norwegian citizens and according to the comparison sheets, 59 cases (77%) resulted in dental identification established, while another 4 (5%) cases had the dental identity conclusion probable. Here specific description of the concordant detail that lead to the conclusion and eventual explanation of possible excluding details should be given. However, in most cases, only standard phrases were given and often only an excuse for bad examining conditions. Excluding details were often not explained and if to be taken seriously, no identification should have been made.

As a conclusion it can be said that in almost none of the cases would the dental reports from Thailand have been accepted by normal quality control in Norway.

This presentation may contribute to a better understanding of how to make ante- and postmortem dental reports and comparison reports on Interpol forms. A possible quality improvement in the use of these forms as well as other types of forms may also be the goal.

**Identification, Dental Forms, Tsunami**

## F38    Identity by Teeth:  Odontological Identification in Milano

*Danilo De Angelis, DDS\*, Cristina Cattaneo, PhD, Daniele Gibelli, MD, and Marco Grandi, MD, LABANOF, Laboratorio di Antropologia e Odontologia Forense, V. Mangiagalli, 37, Milan, 20133, ITALY*

After attending this presentation, attendees will see the importance of odontological methods in personal identification, through an analysis of over 400 cases of unknown decedents examined at the medicolegal institute in Milan.

This presentation will impact the forensic science community by showing the importance of odontological methods of identification in comparison with genetic testing.

The reality of decedents without valid identification is actually not well known in Europe.  The lack of information concerning the problem of unknown decedents and the absence of common guidelines in order to make easier the recording of data useful in personal identification procedures are the main limits in the attempt at identification.  In Italy, as in other European countries, there is no official data on the quantity of unknown decedents only in Milano in the last twelve years, 80 cadavers/human remains still remain unidentified.  This is mainly due to the lack of an antemortem and postmortem database concerning missing persons and unknown decedents respectively.  The Ministry of Internal Affairs is drafting a law for this purpose but the project is only at its beginning.

In theory, when a possible match is performed between an antemortem and postmortem profile, then genetic testing, odontological, and anthropological methods can be used for identification.  Fingerprint analysis is reliably and easily performed, but requires that the fingerprint profile of the individual is recorded, which occurs in Italy only if the subject is arrested by police forces.  Genetic methods allow one to perform a specific identification, but they need a parent in direct line or DNA material from the missing person.  Anthropological methods are based on the comparison between bone structures and are easy to be performed; however, with the frontal sinus comparison, the final result is difficult to evaluate and cannot always supply a definite judgment.  Odontological methods are based on comparison between the dental profile from the missing person and that of the unknown decedent.  They are easily performed and usually give satisfactory results, thanks to the wide inter-individual variability in shape, position, pathologies and treatment characteristics.  This presentation aims at illustrating the importance of odontological methods in personal identification through an analysis of over 400 cases of unknown decedents examined at the medicolegal institute in Milan.  Since 1995 LABANOF, Laboratorio di Antropologia e Odontologia Forense, has recorded data of unknown decedents who underwent autopsy at the Insitute of Legal Medicine.  The number of unknown decedents between 1995 and 2007 amounts to 420 individuals, 3% of all dead people which underwent postmortem examination during the period of observation (13814 subjects).  Among the 420 cases, 64% reached a positive identification, whereas 17% had no name (others have "aka"s which have to be assessed by the police, as in the case of illegal immigrants who give false identification).  For the cadavers identified which were badly preserved, odontological methods were the most successful (25%), followed with anthropological ones (23%) and then by DNA (5%).  The reason behind this is that clinical and non-clinical dental antemortem data is easily recovered and the methods are quicker and cheaper.  This presentation has therefore strengthened the importance of odontological methods as a valid and reliable personal identification procedure.  Four cases in particular are presented: the first in which a skeleton found at the bottom of a lake could be identified only by dental restorations visible in an antemortem thoracic x-ray; a second case of a burned body where odontological methods cleared up a genetic "error"; and two cases in which the decedent had no clinical dental data but dental superimposition with an antemortem photograph was successful in one case and visibility of restorations in non clinical pictures came in useful in the other.

**Forensic    Odontology,    Forensic    Anthropology,    Personal Identification**

## G1 Exertional Collapse in a Youth With Hemoglobin SE

Elise F. Arbefeville, MD*, and Vernard I. Adams, MD, Hillsborough County Medical Examiner Department, 401 South Morgan Street, Tampa, FL 33602

After attending this presentation, attendees will understand one of the potentially lethal sequelae of hemoglobinopathy SE.

This presentation will impact the forensic community by helping forensic pathologists recognize occurrences of exertional death in compound heterozygous SE hemoglobinopathy.

Hemoglobin S is the most common abnormal hemoglobin. It occurs in 10% of North Americans of West African descent. Hemoglobin E is the second most frequent abnormal hemoglobin and is found in persons of Southeast Asian ancestry. Because of the wide geographic separation of the original epicenters for these genotypes, the double heterozygous and manifesting as hemoglobinopathy SE is uncommon with less than 30 cases reported. Hemoglobinopathy SE is typically asymptomatic. A twelve-year-old American boy was participating in football practice in the sun in summer when he collapsed ill on the ground. When the paramedics arrived, he was alert, hypotensive and in sinus tachycardia. His temperature was 97.3 degrees Fahrenheit. During transportation to the hospital his heart was unstable and several sternal rubs were performed. He initially responded to the treatment but his heart rate dropped suddenly. He became unresponsive with pulseless electrical activity followed by asystole. Resuscitation efforts started by the paramedics and continued in the Emergency Room were unsuccessful. On gross examination the spleen, lungs, brain, liver, and heart were unremarkable. Microscopically the cerebellum, heart, and kidney had sickled erythrocytes. Hemoglobin electrophoresis revealed 0.9% hemoglobin F, 57.4% hemoglobin S, 34.2% hemoglobin E, and 7.5% other. Follow-up investigation determined that the decedent's father was of West African ancestry and his mother had Thai ancestry. This is an example of exertional collapse in a person with hemoglobinopathy SE with a clinical presentation similar to that sometimes observed in persons with sickle cell trait. The pathophysiology of sickling in persons heterozygous for hemoglobin S is discussed with particular reference to the mitigating and aggravating effects of other abnormal hemoglobin haplotypes.

**Hemoglobinopathy SE, Exertional Collapse, Sudden Death**

## G2 Machinery-Related Occupational Death: The Relevance of Workplace Investigation and Antemortem Radiological Data in Forensic Reconstruction

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After attending this presentation, attendees will have a clear example of the utility of radiological data and workplace investigations in reconstructing work-related deaths.

This presentation will impact the forensic community by demonstrating the importance of taking into consideration radiological data (x-rays, computed tomography or nuclear magnetic resonance) obtained at hospital admission and of performing a detailed work-place investigation when a work-related incident must be investigated.

A "work-related death" is defined as "A person who was fatally injured as a result of, or who died of a fatal condition caused by, exposure to their own or others' activity or work factors; or who was fatally injured whilst travelling to or from work."

In 2002, 1,478 Italian workers died from work-related injuries. In the same year 5,475 United States workers died due to traumatic injuries related to their work.

There are many potential contributing factors to any work-related incidents, including aspects or characteristics of the working systems, the equipment and material used, the environment, and the worker. For these reasons it is very important to verify if any malfunctioning equipments or any lack in safety measures have played a role in the occupational injuries.

In many cases reconstructing the event can be difficult because of the lack of detailed information about the circumstances of the incident and the design description of the machinery. Furthermore, the victim may not die at the workplace, but is admitted to the hospital and undergoes neurosurgery. In such cases neurosurgeons, removing skull fragments and generating new fracture lines, can complicate forensic examination and sometimes prevent a clear identification of the etiology of the lesions.

A case of a 40-year-old man who was involved in an injury while performing his job in a manufacturing industry will be presented. The victim, found unconscious by a colleague, was immediately transported to the general hospital. In the emergency room he presented with severe cranial trauma with bilateral skull fractures, a subarachnoid hemorrhage, and multiple cortical and intraparenchymal contusions. Despite urgent craniotomy and neurosurgical treatment the man died due to increased intracranial pressure.

Forensic autopsy revealed:
- the laceration of the right ear;
- a curved surgical sutured incision at the left side of the head;
- a lack of part of the left parietal bone due to the craniectomy;
- a linear fracture of the right parietal bone;
- massive subarachnoid and fourth ventricular hemorrhage;
- hemorrhagic necrosis of the pons and medulla oblongata.

There were no witnesses watching the accident and the pictures of the work place did not help the reconstruction. Moreover, the findings

collected at autopsy did not allow investigators to establish whether the skull had been struck by a blunt object or had hit the ground violently, preventing a clear identification of the etiology of the cranial fractures.

To analyze the morphology of the fractures and their location a three-dimensional (3D) reconstruction (surface shaded display, SSD) based on CT scans performed at admission to the emergency room was employed. The analysis revealed a depressed skull fracture involving the left sphenoid and temporal bones with penetration of bone fragments in the left temporal lobe.

With the new information gained from the 3D-CT reconstruction of the skull, a second work-area investigation was performed. The fit-matching analysis between the components of the machinery and the depressed skull fracture permitted to identify a metal parallelepiped as the cause of the cranial staving and to reconstruct the event.

This case underlines the importance of taking into consideration radiological data (x-rays, computed tomography, or nuclear magnetic resonance) obtained during hospital admission and of performing a detailed work-place investigation when a work-related incident must be investigated and reconstructed.

**Machinery-Related Occupational Death, Forensic Radiology, Work-Place Investigation**

## G3 Impetigo Contagiosa Simulating Non-Accidental Injuries in a Pregnant Woman Using Intravenous Drug

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After attending this presentation, attendees will understand the possibility of pitfalls when distinguishing between pathological and traumatic injuries.

This case presentation will impact the forensic science community by emphasizing the possibility of these pitfalls when distinguishing between pathological and inflicted injuries.

This case confirms that for subjects using intravenous drugs, a fulminating course of endocarditis by *Staphylococcus aureus* that involves the left cardiac valves in association with systemic embolism of cutaneous vessels may cause dermal lesions simulating non-accidental injuries.

Even though it is well known how the use of intravenous drug increases the risk of endocarditis, available data about the clinical aspects related to the involved site and bacteriological characterization seem to be controversial. *Staphylococcus aureus* represents the most frequently implicated microorganism (i.e., 76% of cases) that significantly impacts the tricuspid valve. Different from the other etiologic forms, the endocarditis by *S. aureus* generally starts with symptoms of sepsis and pulmonary embolism linked to a past use of intravenous drug which defines the so-called "diagnostic triad" of the tricuspid valve endocarditis. Cardiac insufficiency and neurological signs are not usual symptoms. In endocarditis cases resulting from *S. aureus*, a fulminating course has been observed only if the left cardiac valves were involved, with systemic embolism and/or cardiac decompensation. The course is favorable in the remaining cases.

**Case:** A young woman, at the 32.2 week of amenorrhea was assisted by first aid and admitted to the hospital with a diagnosis of "labor of preterm fetus." At the clinical exam, the woman was in a very bad general condition and not awake. She had widespread signs of acupuncture, ecchymoses and bruises in the forearms, bruises and scrubs on both the thighs and the vulva. The fetus was in cephalic presentation and the membranes were broken and very bad smelling. The woman was assisted during labor. However, the fetus, a male weighing 1,530g, was terminal. Immediately after the labor, the woman exhibited cyanosis, marked hypocapnia, hypotension, and hypothermia. She was transferred to the intensive care unit where she arrived unconscious with tachypnea, tachycardia, hypotension, metabolic acidosis, hyperkalemia, and hypercreatinemia. Despite intubation, a sudden bradycardia arose evolving into asystole after about four hours. Resuscitation was attempted but the patient died by electro-mechanical cardiac dissociation. The external exam of the decedent showed extended bruises and abrasive injuries on the thighs and on the vulva, resulting in the hypothesis that the woman could have been a victim of violence. The judicial authority, considering the clinical evolution of the patient and the hypothesis of personal violence, ordered the autopsy of the woman and fetus.

The autopsy and histological examinations revealed tricuspid valve acute vegetating endocarditis by *S. aureus*, multiple septic pulmonary, renal, encephalic, cardiac and cutaneous emboli, impetigo contagiosa causing apparent cutaneous abrasions, ecchymoses, and consumption coagulopathy. Similarly, the fetal autopsy showed that the cause of death was a sepsis by *S. aureus*.

This case emphasizes the possibility of pitfalls in distinguishing between pathological and traumatic injuries.

**Impetigo Contagiosa, Non-Accidental Injuries, Cutaneous Emboli**

## G4 HPLC Analysis of Benzocaine in "Green Products"

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After attending this presentation, attendees will understand some principles of analyzing condoms that contains benzocaine as a desensitizer. Such a condom is commonly known as a "Benzocaine condom." Benzocaine is a compound that is prepared from 4-aminobenzoic acid and ethanol. It is also the active ingredient in many over-the-counter anesthetic ointments and is indicated for general use as a lubricant and topical anesthetic on intratracheal catheters, pharyngeal and nasal airways, sigmoidoscopes and vaginal specula.

A benzocaine condom is a completely unique condom in itself. These condoms have a small amount of benzocaine lubricant cream in the tip. This cream helps to disperse the heat of the body. The main role of benzocaine is to desensitize the tip of the penis and prolongs the act of lovemaking between couples. Benzocaine binds to sodium channel and reversibly stabilizes the neuronal membrane which decreases its permeability to sodium ions. Depolarization of the neuronal membrane is inhibited thereby blocking the initiation and conduction of nerve impulses, thus making the sexual encounter last longer.

The condom is a widely used mechanical barrier contraceptive. It is one of the oldest methods of birth control. They are available over the counter as a non-prescription product and are procured very easily by sexual offenders. Sexual offenders often use condoms in the commission of sexual assaults in order to prevent identification through deposited biological material. Even the detection of DNA is inhibited in cases of sexual assault involving condom use. In such circumstances trace evidence, including condom lubricant residues viz. PEG, PDMS, benzocaine, etc. provides the crucial associative evidence. The seminal fluid residue containing sperm, proteins, blood grouping factors, and DNA helps in identification of sexual assault offenders. However, perpetrators of sex crimes using condoms during the commission of sexual assaults prevent identification through deposited biological

material. However, when assailants use condoms, they leave behind other valuable evidence.

**Benzocaine, HPLC, Condom**

## G5 Child Deaths Due to Television Falls With Injury Patterns That Mimic Child Abuse

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After attending this presentation, attendees will have learned about two cases illustrating the types of injuries sustained when televisions fall on small children and their similarity to inflicted child abuse trauma.

This presentation will impact the forensic community by explaining how blunt trauma secondary to falling televisions is occasionally reported in the clinical literature; however, descriptive reports of the patterns of such injuries are limited and such cases may mimic those considered "typical" of inflicted trauma.

Data from the CDC indicate that accidents and inflicted trauma account for 33 percent and 5-8 percent of childhood deaths, respectively. Blunt trauma secondary to falling televisions is occasionally reported in the clinical literature, however, descriptive reports of the patterns of such injuries at autopsy is limited. Data from the clinical literature indicate that under such circumstances, blunt head trauma is far more common than blunt chest or abdominal trauma. The severity and patterns of injury identified in such cases may mimic those considered 'typical' of inflicted trauma. As such, careful integration of data collected from the death scene (including witness statements), from hospital records, and all components of the autopsy is necessary to ensure accurate and defensible determination of cause and manner of death. Two cases will be presented to illustrate the types of injuries sustained when televisions fall on small children.

Case #1: A 13-month-old male was at his aunt's house, playing with other children. A family member heard a loud crash, after which he observed a 21-inch cathode ray tube-type television lying on the child's head. Emergency Medical Services (EMS) was summoned, and the child was transported to the nearest Emergency Department (ED). After initial evaluation, he was admitted to the Intensive Care Unit (ICU). A computed tomography (CT) scan revealed left-side calvarial skull fractures, left orbital skull fracture with slight proptosis, laceration of the left transverse dural venous sinus, and expansile intracerebellar hematoma, for which he underwent suboccipital craniotomy. Despite supportive measures, he expired 15 days after hospital admission. Pertinent autopsy findings included posterior scalp abrasions; frontotemporoparietal and occipital scalp and subgaleal hemorrhages; simple linear, minimally displaced fractures of the left lateral aspect of the frontal bone and orbital shelf; complex comminuted fractures of the left sphenoid and temporal bones; extensive comminuted fractures of the squamous portion of the occipital bone; subdural and subarachnoid hemorrhages; cerebral edema; cerebellar sequelae of neurosurgical evacuation of intraparenchymal hematoma; bilateral optic nerve sheath hemorrhages; no retinal hemorrhages. Evaluation of 3-dimensional reconstructions of the admission head CT scan demonstrated that many of the fractures identified at autopsy were altered (and thus appeared worse) by brain swelling, and sutural diastasis along a Mendosal suture. The cause and manner of death were certified as *blunt head trauma* and *accident*, respectively.

Case #2: A 32-month-old female was at home with her father and four other children; she was unsupervised while watching television.

The father heard a crash, after which he entered the room and found the child face-up on the floor with a 27-inch cathode ray tube-type television on the floor next to the child. EMS were summoned, the child was transported to a local ED, and admitted to the ICU. An admission CT scan demonstrated complex comminuted multifocal left-side skull base fractures, epi- and subdural hemorrhages, massive cerebral edema with midline shift, and brainstem hemorrhage. Despite supportive measures, her neurologic condition rapidly declined, and a determination of brain death was supported by clinical evaluation. She died two days after hospital admission. Pertinent autopsy findings included bilateral bulbar and palpebral conjunctival ecchymoses; left frontal scalp contusion; frontal and occipital subgaleal hemorrhages; large right epidural hematoma; cerebral edema with bilateral uncal herniation; fragmentation of the cerebellar folia; multifocal cerebral and brainstem hemorrhages; left periorbital soft tissue hemorrhage; scant right perioptic nerve sheath hemorrhage; no retinal hemorrhages. The cause and manner of death were certified as *blunt force injuries to the head* and *accident*, respectively.

**Child Abuse, Accident, Television**

## G6 Application of Forensic Engineering for the Reconstruction of Manner of Death: A Nautical Accident

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Attendees of this presentation will be presented with a case where the application of forensic engineering helped in reconstructing manner of death and mode of lesion production in a nautical accident.

This presentation will impact the forensic community by showing how the application of forensic engineering to cases may result in obtaining more precise data concerning the reconstruction of events.

In forensic pathology it is sometimes necessary to reconstruct the manner in which a victim fell or was hit, stabbed, or shot in order to verify the compatibility between the pattern of distribution of lesions and the dynamics of the lethal event. Reconstruction aids in acquiring information which may help in determining homicide, accident, or suicide. More and more in these cases, forensic engineering assists the forensic pathologist. This case shows the importance of forensic engineering in the reconstruction of events. The case concerns an unmarried couple on a boating trip. One morning the man woke up to find his partner overboard in the water tied at the waist by a security rope. He later reported that she must have fallen in the water during the night, when it had been her turn to steer and check on the boat. The woman underwent postmortem examination, which showed typical signs of drowning, such as foam in the airways, overinflated lungs, and water in the stomach. Authorities initially classified the death as accidental. The woman's family remained suspicious that the partner was responsible for the death since he had recently been made the sole beneficiary of her will. Their accusations led to the exhumation of the corpse and a new autopsy was performed, which highlighted typical signs of blunt trauma, such as bruises distributed to the head, right thorax, right hand and shoulder, back, and linear abrasions on the abdomen.

Microscopic analysis of the lungs showed signs of drowning, such as oedema and expansion of the alveoli. Unfortunately no diatoms could be found (only fragmentary). The cause of death was identified as mechanical asphyxia by drowning. Although the cutaneous signs of blunt trauma observed during the postmortem examination could not

justify death by a traumatic cause, they could have been the signs of an aggression which may have caused the fall of the victim into the water. The main question was: could a simple fall from that boat explain the pattern of lesions (anterior and posterior), or did they indicate an aggression?  Initial experiments were performed with a dummy and a boat of the same model.  Different manners of precipitation in different positions were then simulated, which provided the first general data concerning the mechanisms of the fall.  A more precise analysis was then conducted with computer-simulation software in order to obtain more reliable data concerning the physical characteristics of the boat and dummy model as well as the mutual interactions between the two.  After recording every physical characteristic which may have had importance in the reference system and the virtual reconstruction of the environment, different simulations of the fall were reconstructed.  The position of skin lesions were considered as points of contact between the body and boat during the fall.  Three hypotheses of falling were then considered and simulated.  In the first case, the victim was facing the sea, in the second she had the sea to her right, and in the third she had the sea behind her.  In the first case, the simulation was concordant with all the lesions described but for the bruise on her back.  In the second case the fall could explain only the lesion on her right hand.  The third type of fall explained all lesions.

Results showed therefore that the pattern of lesions could be consistent with an accidental fall and may not have necessarily been caused by an aggression.  This experience strengthens the importance of forensic engineering in the reconstruction of events.

**Forensic Pathology, Forensic Engineering, Nautical Accident**

## G7  Homicide, Suicide, and the Missing Mother: The Mysterious and Tragic Deaths of a Latino Family

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After attending this presentation, attendees will have a better appreciation of the importance of joint investigation of complicated cases involving skeletonized and decomposed remains by forensic pathologists and anthropologists.  In addition, the importance of proper recovery techniques at a burial site to insure complete recovery of all skeletal remains and associated evidence will be discussed.  Strong emphasis will be placed on the importance of DNA maternal and fraternal posterior testing when known antemortem DNA comparative samples of a deceased parent are unavailable.

This presentation will impact the forensic community by enforcing among forensic scientist to utilize a group approach when dealing with complicated homicides involving decomposed and skeletonized remains.  Utilizing of individuals representing various forensic specialties can greatly increase the rate of success in an investigation.  Use of anthropological skeletal markers such as parturitional pitting of the pelvic bones can be very useful in possibly determining the status of a female as one who has given birth to children vs. a woman who has not given birth to a child.  Such information can be extremely useful to law enforcement when trying to identify a female victim.

In late March of 2007 a gruesome discovery was made by police in Frederick, MD as they entered a local residence.  Discovered in the house was the body of an adult Latino male who was suspended by a noose ligature along the stairwell leading up the upstairs level of the residence.  A continued search of the residence led to the discovery of four children, ages one, three, four and nine.  All four of the children were found deceased and in an advanced state of decomposition.  One pair of the children was discovered in a single bed covered by blankets, and the other pair in a single bed in a separate bedroom covered by blankets.  Placed above the bodies of the deceased children in each room were religious pictures.  An extensive search of the residence by police failed to locate the mother of the children.  Autopsy and forensic examination of the adult male who was identified as the father of the children determined the cause of death to be asphyxiation and the manner of death suicide by means of hanging.  Autopsy results for three of the children found death to have resulted from suffocation and the fourth child to have died as the result of blunt force trauma.  All four deaths of the children were determined to be homicides at the hands of their father. Initially it was thought that the reasoning behind the murder and suicide by the father was that he was despondent over his wife being kidnapped, or possibly leaving him for another man.

As these seemingly senseless killings shocked the community, police were baffled as to the whereabouts of the missing mother.  Various speculations surfaced in the news media concerning the disappearance of the mother.  Some individuals suspected she abandoned her family and ran off with another man, others claimed that she had been abducted by a Latino gang as retribution for not paying them for illegal assistance she may have received in entering the US.  Reports had even been received from her sister who lived in the same community that she had returned to her native El Salvador.  The case of the missing mother and the tragic deaths of the father and children received so much media attention that the FBI was called in to assist in the investigation.  Law enforcement officials were contacted in El Salvador who later reported possible sightings of the mother in their country as well as bordering Honduras.  The plausible leads and high profile nature of the case prompted the FBI to send agents to El Salvador to investigate the sightings.  Based on the primary evidence gathered by law enforcement it was believed that the mother had been kidnapped.  An intensive national media blitz was conducted to provide leads to police and the FBI in order to locate the mother.

Human skeletal remains were discovered at a clandestine burial site located in the same county in which the deceased family had resided almost a year after the horrific discovery of the murdered children and their father's suicide.  The grave was located by a local real estate agent who was conducting a survey of a four acre parcel of land located along the edge of a major highway.  While walking the property the real estate agent discovered a skull and partial lower limbs exposed within the grave.  Also discovered lying directly next to the grave was a snow shovel and a large digging pick. The Office of the Chief Medical Examiner was immediately called to the scene where the remains were carefully recovered.

During the initial recovery of the remains anthropological analysis determined that the remains represented a  Mongoloid / Hispanic female who was in her early twenties at the time of death.  Examination of the bones of the pelvis at the scene also determined that the deceased had given birth vaginally to multiple children as indicated by the presence of deep and extended parturitional pitting along the ventral surfaces of the pubic bones of the inominates.  Several jewelry items were located with the remains which included a gold religious symbol common to Central American coastal countries. Examination of the gravesite revealed it to be an incomplete burial, as the body was never fully covered possibly due to its close proximity and visibility from the highway, and the non-retrieval of the digging tools.

Anthropological examination of the skeletal remains found them consistent with the biological profile for the missing mother.  Due to the lack of antemortem dental or radiographic records, DNA analysis was conducted on the skeleton.  Positive identification of the remains was established by comparing the postmortem DNA profiles obtained from the children and the husband to that obtained from the bones of the deceased in question.  No skeletal injuries were noted on the remains therefore cause of death was undetermined but manner signed out as homicide. Continued investigation of the case revealed that the woman and her husband had been having marital problems which arose from the wife seeing other men. As a result of the martial unrest it is believed that

the husband murdered his wife and buried her body along the interstate. Possibly despondent over his actions the father, knowing he would be imprisoned and the deleterious affect it would have on his children, he chose to kill the children and then commit suicide.

**Asphyxia, Buried Remains, Parturitional Pitting**

## G8 Sudden Death in Epilepsy: A Review of 51 Consecutive Cases

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After attending this presentation, attendees will be able to list the risk factors for sudden death in epilepsy, discuss pathophysiological mechanisms for sudden death in epilepsy, and address the role neuropathological examination in epilepsy cases.

The presentation will impact the forensic community by providing a broader understanding of the role of seizures in sudden death as well as the role of detailed neuropathological examination in characterizing such cases. The presentation will also identify two additional subgroups of epilepsy patients and provide attendees of an enhanced understanding of causes of death in epilepsy patients in general.

Sudden unexpected death in epilepsy (SUDEP) is a well-known but poorly understood phenomenon. While certain risk factors are consistently associated with SUDEP, the pathophysiological mechanism for sudden death remains speculative. Autopsy data from 51 consecutive cases with a history of "seizures" and who underwent complete autopsy, including toxicology and neuropathological examination were reviewed. A board-certified neuropathologist completed the neuropathological examination in 50 of the 51 cases. Of 51 cases, 24 (47%) met criteria for SUDEP (history of epilepsy, sudden unexpected death, no other cause of death, no status epilepticus). Of the cases meeting criteria for SUDEP, 15 (63%) were male and nine (37%) were female, with a mean age of 32.4. Fourteen were found in bed, none were found outside, and eight were lying prone. Seven (29%) had evidence of tongue biting. Fourteen were treated with a single antiepileptic drug. Two were receiving polytherapy. General autopsy revealed pulmonary edema in 17 (71 %) cases. Twelve of 19 cases had childhood onset of epilepsy. Neuropathological examination revealed significant abnormalities in 67%. Among these were remote contusions, vascular malformations, hamartomas, mesial temporal sclerosis, and migration disturbances. Of cases excluded from the SUDEP category, two groups were apparent: one with complex neurological disorders in children complicated by seizures (CND-S), and the second with atherosclerotic cardiovascular disease in older decedents complicated by seizures (ASCVD-S). No acute cause of death was apparent in a number of these cases, raising the possibility that seizures could have played a role. In conclusion: 1) cases discussed indicate general SUDEP risk factors consistent with the published literature; 2) the percentage of cases with significant neuropathological findings is higher than indicated in other studies, emphasizing the need for detailed neuropathological examination (formalin fixation and examination by a neuropathologist); and 3) two additional subgroups, ASCVD-S and CND-S, are in need of further study regarding the role of seizures in sudden death.

**Epilepsy, Sudden Death, Seizures**

## G9 Spontaneous Coronary Artery Dissection – An Isolated Eosinophilic Vasculitis?: Report of Two Sudden Death Cases

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After attending this presentation, attendees will understand two cases of spontaneous coronary artery dissection, recognize them as a cause of sudden death, and discuss the role of an adequate gross recognition and histological examination with emphasis on the presence and significance of the eosinophilic inflammatory infiltrate that is frequently associated to this disorder.

This presentation will impact the forensic community by increasing the awareness of the existence of this rare natural disorder and demonstrating its pathological characteristics emphasizing in the gross recognition and histological presentation.

Spontaneous dissection of the coronary artery is a rare entity. It has an increased prevalence in women, especially in the peripartum state. It is defined as hemorrhagic separation of the media of the coronary artery with creation of a false lumen, in the absence of chest trauma, extension of aortic dissection or iatrogenic trauma.

The first case involved a 55-year-old woman with no personal or family history of heart disease. History was also negative for systemic disease, recent trauma, or drug abuse. She was last seen in her usual state of good health a few hours before her death. She was found unresponsive by family members at her apartment where she was pronounced dead after unsuccessful resuscitative measures.

At autopsy the decedent was 167 cm tall and weighed 72 kg. Externally there were no signs of natural disease or trauma. The heart weighed 370 g without ventricular hypertrophy or gross ischemia. The coronary arteries were free of atherosclerosis and had a normal distribution. The left anterior descending coronary artery (LAD) had a focal dissection within the media with a hematoma surrounding and compressing the wall causing total occlusion of the lumen. The total length of the dissection was 2 cm, and it started 3 cm from the origin of the LAD.

The second case involved a 43-year-old woman whose medical history was relevant for back pain, occasional episodes of tachycardia and shortness of breath. She had no history of recent trauma or drug abuse. She was in her usual state of health when she complained of increased back pain and shortness of breath. She was taken to the emergency room by family members but was pronounced dead on arrival. At autopsy the decedent was 165 cm tall and weighed 67 kg. Externally there were no signs of natural disease or trauma. The heart weighed 310 g without ventricular hypertrophy or gross ischemia. The coronary arteries had a normal distribution with minimal atherosclerosis. The LAD showed a focal dissection within the media with a hematoma compressing and occluding the lumen of the artery. The total length of the dissection was 1.5 cm at the distal third of the LAD.

A common histological finding for both cases was a dense focal infiltration of the adventitia and the outer media of the dissected coronary artery by inflammatory cells of predominantly eosinophilic granulocytes with a few lymphocytes and mononuclear histiocytes. Polymorphonuclear granulocytes were infrequent. The inflammation did not involve the inner media or intima. The non-dissected portions of the LAD, the rest of the coronary arteries and the myocardium were free of inflammatory infiltrates in both cases. No myocyte hypertrophy, myocardial scarring, or small vessel disease was present.

Spontaneous Coronary Artery Dissection is a rare entity whose precise incidence, etiology and pathogenesis have not been clearly established. Periadventitial and medial wall eosinophilic inflammation have been commonly observed, generating the hypothesis of an underlying localized inflammatory or vasculitic process that predisposes to this condition. This primary process could cause weakening of the arterial wall and subsequent dissection. However it has also been proposed that such inflammation could be a consequence of dissection, rather than its cause.

These two cases illustrate that a detailed examination of not only the affected coronary artery but also the rest of the vasculature and myocardial tissue is essential to identify and understand this process. In order to clarify the pathogenesis of this entity, it is necessary to perform future studies including cases of non-spontaneous dissection of the coronary artery. These cases are presented and discussed with a review of the literature available to date.

**Spontaneous Coronary Artery Dissection, Sudden Death, Eosinophilic Inflammation**

## G10 Fibromuscular Dysplasia of Pulmonary Arteries: Report of Two Cases

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After attending this presentation, attendees will learn of a case showing complications of fibromuscular dysplasia (FMD) (aneurismatic dissection, arterial obstruction) most likely triggered by chest trauma and by possible cardiotoxicity due to association local/general anesthesia.

FMD is a non-atherosclerotic and non-inflammatory vascular disease, with a familiarity of 10% (inheritance dominante autosomica), and is characterized by fibrous or muscular or both types proliferation subverting normal architecture of the arterial wall. Etiology of FMD is unknown although various hormonal and mechanical factors have been suggested. Fibromuscular dysplasia generally affects women (94%) in their fertile age; it is frequently associated with pregnancy or hyperestrinism, but FMD can occur in any age, infancy included.

Clinical manifestations of FMD depend on involved arterial segment, histological type, and complications (obstructions, aneurysm rupture; embolism; sudden death). FMD commonly affects renal and carotid arteries, and less frequently it's observed in other small and medium arteries; pulmonary localization is rare. Prevalence of symptomatic renal FMD is about 4/1000 cases, twice as to that observed in carotid arteries. Histologically, FMD has been classified into three distinct types: intimal fibroplasia; fibromuscular medial dysplasia (medial hyperplasia, perimedial fibroplasia), and periarterial (adventitial) fibroplasia. Angiographic classification includes multifocale type, related with histological variant "medial fibromuscular dysplasia"; tubular and focal types, both no related with specific histological type.

In this study, two cases of unknown FDM involving pulmonary arteries are described. Clinical manifestation occurred in one case following a road accident related trauma and, in another case, following an anaesthetic induction and local anesthesia before surgical procedure.

**Case 1:** A 52-year-old obese man while driving a car got into an accident and suffered severe multiple trauma. He was taken to the Emergency Room where he presented coherent and breathing (SpO2 92%), with SBP/DBP 150/90 and CF 92b/m.

Chest x-ray showed several rib fractures on the right side, associated with bilateral hydrothorax, upper pneumomediastinum; mild right pneumothorax. After the first day the patient refused hospital care and discharged himself but a few hours later, he went to another hospital due to persistent pain. When he arrived was mildly dyspnoic and had bilateral basal pleural effusion. During his second hospitalization, he received antibiotics, anti-thromboembolic, anti-hypertensive, and gastroprotective therapies, and had progressive improvement of his clinical conditions. Six days after release, the patient suffered cardio-respiratory arrest and was not responsive to rescue procedures. Autoptic histological finding were mainly in the lungs that showed conspicuous bilateral pulmonary hemorrhage associated with perimedial fibroplasia variant of FDM, with aneurismatic and dissecant patterns, in lack of pulmonary embolism.

**Case 2:** A 31-year-old female patient was scheduled for rhinoplasty. Presurgical hematochemical and cardiovascular examinations were normal and anesthesiological risk class was ASA1. Twelve minutes after general anaesthesia induction and immediately after infiltration of nasal mucosa with mepivacaina and adrenalina, a rapid decrease of both oxygen saturation and cardiac frequency occurred until there was irreversible cardiocirculatory arrest, and no response to rescue procedures. Autoptic histological finding were mainly in the lungs that showed vascular congestion and acute focally hemorrhagic edema, associated with FDM, perimedial fibroplasia type. Chemical-toxicological analysis for research of Mepivacaina levels showed non-toxic concentration.

**Conclusions:** In both described cases death was referable to complications of FMD (aneurismatic dissection, arterial obstruction) most likely triggered by chest trauma in the first case and by possible cardiotoxicity due to association local/general anestesia.

**Fibromuscolar Dysplasia, Pulmonary Arteries, Histopathology**

## G11 Sudden Death From Arteritis Involving a Surgically Repaired Coronary Artery - Right Atrium Fistula

*Marc A. Krouse, MD\*, Tarrant County Medical Examiner, 200 Feliks Gwozdz Place, Fort Worth, TX 76104-4919*

After attending this presentation, attendees will be informed of the possibility of late complication of a repaired congenital coronary artery anomaly by an independent pathologic process.

This presentation will impact the forensic science community by revealing an unusual complication of surgically treated congenital cardiac malformation, specifically coronary artery - right atrium fistula.

After attending this presentation attendees will appreciate that an anomalous coronary artery (coronary artery – right atrial fistula), successfully repaired many years prior, may be involved by independently occurring disease processes such as pan-arteritis and may prove a cause of morbidity or mortality despite successful earlier treatment.

The subject of this presentation was an 11-year-old male who had a diagnosis of coronary artery – right atrium fistula some eight years prior. The anomalous vessel was ligated at the distal (right atrium) end and he was followed, without complication, for a period of some two years. He was well and active until the day prior to his collapse and demise with no complaints that could be related to cardiac disease. In early morning hours his family members responded to sounds of distress, he collapsed and began dry vomiting before becoming unresponsive. Resuscitation efforts, including ACLS protocol and emergency department treatment, were unsuccessful and he was declared dead less than two hours after onset. His history of previous surgery was initially reported (incorrectly) as repair of an abnormal right coronary artery.

At necropsy examination the body was normally developed. There were diffuse pericardial adhesions over the anterior and left side of the heart. Serial sectioning of the left coronary artery circulation revealed a

slightly large (4-5 mm) left main coronary artery with a similar size anomalous branch passing posterior to the aortic root between the atria. In this area the vessel was markedly dilated (up to 2 cm) and filled with layered, clotted blood. The firmer clot had propagated retrograde and gelatinous, acute clot was found throughout the proximal part of the anomalous artery, into and occluding the left main coronary artery. The left coronary artery ostium was also large, some 1 cm. Microscopic sections of the coronary arteries and coronary artery fistula were notable for active pan-arteritis and healed arteritis in the dilated area of the fistula as well as layered blood clot without notable organization. There was no gross or microscopic evidence of ischemic myocardial injury.

The gross appearance of the artery fistula was reminiscent of Kawasaki disease and the pan-arteritis points to a similar pathogenesis of the vascular injury, aneurismal dilation and eventual thrombosis of the injured vessel. His recent medical history included only an episode of acute sinusitis with a four day course of an unknown prescribed medication, but in interviews with family a previous episode of a viral illness some four months prior was elicited.

This case study is presented to inform forensic and/or pediatric pathologists of the possibility of a late complication of a successfully repaired anomalous coronary artery, presumably by an immune-mediated vascular injury indistinguishable from typical Kawasaki disease.

**Coronary Artery Fistula, Arteritis, Sudden Death**

## G12  Ephemeral Petechial-Like Spots in a Victim of a House Fire

*Russell T. Alexander, MD\*, and David R. Fowler, MD, Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201*

After attending this presentation, attendees will be familiar with a case of short-lived petechial-like spots on a woman who died due to inhalation of soot and smoke in a house fire.

This presentation will impact the forensic community by discussing the occurrence of transient petechial-like spots in fire related deaths.

A 33-year-old black female was found deceased within her apartment after a house fire. Per report, she had been drinking alcohol that evening with a girlfriend. At approximately 3:25 a.m., her daughter heard the fire detector within their apartment go off. She opened her bedroom door, saw thick black smoke, and then exited the apartment through her bedroom window. The fire department responded and was informed by the daughter that no one else was in the apartment because she thought her mother was still out drinking. The fire department extinguished a "small" fire in a loveseat located on the east end of the living room. Extensive soot was deposited throughout the residence except for the daughter's bedroom. The decedent was found "hiding" behind a chair in the northwest corner of the living room. Her keys were found underneath the burned loveseat. An ashtray with four cigarette butts was on an end table within the living room. An investigation of the fire revealed no evidence of foul play.

At autopsy, soot was densely deposited on the face, within the nares and on the tongue. Less dense soot was deposited over much of the body. Partial thickness burns involved approximately a third of the body surface area. Internally, dense soot was deposited in the airways. No thermal fixation was noted to the airways. The level of carboxyhemoglobin in iliac blood was 62.2%. The iliac blood alcohol content was 0.14 mg/dl. No other drugs were detected on a comprehensive drug screen.

Washing of the body revealed a petechial-like rash on the eyelids, face, shoulders, and back in areas where the epidermis was wiped away during cleaning. The spots appeared to have a follicular or peri-adnexal distribution. A similar though quite subtle pattern of spots was on adjacent areas where the epidermis was intact. Reexamination of the body two hours later revealed that the petechial-like rash had often faded

to a blotchy red-purple area of drying skin, though some faint spots remained. When intact epidermis along the edges of denuded skin was wiped away at this time, a new crop of petechial-like spots emerged. Two hours later, the second set of spots had faded similar to the first. Wiping away more epidermis made a third round of spots apparent. A histologic section of skin showed congested dermal blood vessels, particularly adjacent to hair follicles.

Conjunctival and facial petechiae are thought to be due to increased cephalic venous pressure resulting in rupture of small blood vessels and extravasation of blood; morphologically similar "Tardieu Spots" are formed in areas of livor mortis when engorged blood vessels in dependent portions of the body rupture (Ely and Hirsch, 1999). A literature review found only a single reference to petechiae in a fire victim (Rao and Wetli, 1988). The petechial-like spots in the present case are not related to lividity since they were equally prominent along the anterior and posterior surfaces of the body. The fading of the spots demonstrates that they are due to a congestive process and not vasculature rupture; a finding confirmed by histologic examination. It is possible that rubbing the skin created physical traction that drew blood into the vasculature further accentuating the pattern. Blood flow out of intact vessels into surrounding tissues caused the spots to fade.

This case demonstrates that inhalation of soot and smoke in a house fire can be associated with congestion of peri-follicular and adnexal blood vessels resulting in a subtle petechial-like rash that will be accentuated by wiping away of the epidermis. Furthermore, rupture of capillaries and venules with extravasation of blood is not necessary for the formation of petechial-like spots.

**Fire, Petechiae, Autopsy**

## G13  Forensic Identification of Microbial Mixtures Via ESI-TOF Mass Spectrometry

*Joshua K. Stone, BS\*, and Raleigh W. Parrott, MS, Federal Bureau of Investigation, CFSRU, 2501 Investigation Parkway, Quantico, VA 22135; Jason D. Bannan, PhD, and Bruce Budowle, PhD, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA; and James M. Robertson, PhD, and Brian A. Eckenrode, PhD, Federal Bureau of Investigation, CFSRU, Federal Bureau of Investigation Academy, Building 12, Quantico, VA 22135*

After attending this presentation, attendees will learn the basics of ESI MS, the basic problems with current identification methods of unknown microbes, and how ESI-TOF can correctly identify microbes based on DNA base composition.

This presentation will impact the forensic community by explaining a novel method of identifying unknown microbes in a complex sample.

The growing threat of bioterror events is a significant problem for the security of individuals worldwide. When an unknown biological agent is released, identification can be delayed due to complexity and number of samples required. Whole genome sequencing (WGS) is a possible solution; however this can be costly for complex mixtures. Targeted methodologies search for specific bacterial agents and can be limited by the requirement to determine if the agent of interest is present within the sample. An alternative to WGS and targeted methodologies was developed by Ibis BioSciences using mass spectrometry (MS). This new MS-based method allows an analyst to determine initially which species are present in the sample, rather than asking if a certain species of bacteria is present. Further delineation is then possible by fine-tuning the assay. The DNA base composition can be determined by mass measurements using high resolution MS, which can detect differences in DNA and allow strains of bacteria to be identified. A primary goal of this research is to determine the level of strain delineation possible amidst other strains of a select microbe using this MS-based method.

Broadly conserved genes in bacteria were selected for amplification with specifically designed forward and reverse primers to *Bacillus*

*subtilis*. Genomic DNA was extracted from *B. subtilis* and amplified using PCR. These selected amplicons were analyzed via Electrospray Ionization Time of Flight (ESI-TOF) MS. Using an integrated fluidics system, DNA samples could be introduced to the ESI source at a high flow rate but then electrosprayed at a slower flow rate to improve resolution. After deconvoluting the information from the mass spectrometer, the organism can be identified by comparison to a library using abundance estimation, joint maximum likelihood, and base composition analysis. The molecular weights from multiple strands, when combined, provide a unique molecular fingerprint which allows an organism to be identified down to the species and strain-level.

A binary set of strains from *B. subtilis* were mixed at various concentration levels to evaluate this MS-based approach in terms of speed and accuracy. An internal mass standard sequence of DNA was used to allow the concentrations of microbial DNA to be calculated after amplification. When using single-stranded oligonucleotides, more than 1200 base compositions could be reported. However, using the complement strand at low concentrations has shown to reduce complexity and error in the data, improving the accuracy of the result.

An expansion and variation of the number of bacterial species and strains tested will occur as time permits for this presentation. As a bioterror event could result in thousands of organisms present in a sample, there will continue to be a need for methods which can select the correct organism, especially in the case of a novel strain for forensic studies.

**ESI-TOF, Unknown Microbes, Base Composition**

## G14 Environmental Scanning Electron Microscopy and Other Techniques in Cutting Crime Investigation: Case Report and Review of the Literature

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After attending this presentation, attendees will understand some principles of investigation of a dismembered body concerning the cause of death and the identification of the tools used to separate arms and legs from the trunk.

This presentation will impact the forensic community by suggesting a novel approach for the analysis of cutting crimes in general and dismembering in particular.

Herein investigators present the case of a 40-year-old female killed by throat cutting and consequently dismembered. She was found cut into 30 pieces inside three plastic bags in a garage.

Dismemberment is the act of cutting, tearing, pulling, wrenching, or otherwise removing the limbs from the trunk of a living or deceased object. It may be practiced upon human beings as a form of capital punishment, a result of a traumatic accident, or in connection with murder, suicide, or cannibalism. After killing the victim, the murderer uses a very sharp cutting weapon (a saw, knife, axe, etc.) to sever the limbs and cut the body into pieces. The operation is generally carried out immediately after the crime, although more rarely a long time may pass between the two events. There are two types of dismemberment that are commonly seen: localized, such as the removal of the head or hands in an attempt to hinder identification of the victim, or generalized at multiple sites (commonly bisection of limbs or disarticulation of the joints) to aid in the disposal of the body. In these cases a new pattern of investigation must support classical techniques to solve the following forensic issues:

- The evaluation of the time since death and of the time since dismemberment. Indeed, exsanguinations and dismemberment of the body prevent an accurate evaluation of lividities and rigidity.
- The identification of the tools used to cut the body. Careful, thorough investigation is a key point to ensure that potential physical evidence is not tainted or destroyed. In particular it is essential to identify any potential sharp cutting weapons at the crime scene. Moreover, when saws are used to cut the body, characteristic tool marks are left on the bone. The nature of the marks depends on the size, shape, width of the saw, and on the sawing action of the user.

Environmental Scanning Electron Microscopy (ESEM) may help in identifying the specific saw that has been used in the act of dismemberment. ESEM can detect and measure different types of striations, paint traces (such as rust inhibitor paints) or metal residues remaining on the bone after the cutting.

In the case presented, the determination of potassium levels in the vitreous humour and their time changes showed that the victim had been killed 20 – 25 hours before the death scene investigation.

The absence of lividities combined to histological and immunohistochemical investigation of the skin let investigators classify the dismembering injuries as non-vital wounds and to estimate the time interval between the death and the dismembering.

However, the most interesting finding was the identification of the tools used to cut the soft tissues and the bones of the victim. Morphological and morphometrical analysis of the skin lesions pointed out that the arms and the legs were cut with a sharp knife, whereas the head was removed from the trunk by a woodworker saw. ESEM analysis determined that the bone injuries were produced by a particular type of saw covered by rust-inhibitor paint.

**Cutting Crime, ESEM, Dismembering**

## G15 Exploration of Non-Cardiogenic Pulmonary Edema With Chronic Opiate Use: Case Studies and Scientific Review

*Marrah E. Lachowicz, MFS\*, University of California Davis, One Shields Avenue, UCDSOM, Tupper Hall 4112, Davis, CA 95616-8643*

After attending this presentation, attendees will learn how to characterize non-cardiogenic pulmonary edema during autopsy caused by opiate use. Additionally, attendees will learn about alternative opiate sources which may lead to cases of non-cardiogenic pulmonary edema. Such cases may present during autopsy and potentially lead to classification of a secondary cause of death or change in the classification of manner of death.

This presentation will impact the forensic community by providing potential answers to cases in which underlying chronic opiate use potentiates mortality. Chronic opiate use may synergistically lead to fatal pathology not readily recognized when secondary to diseased states in the lung. The presentation focuses on the reliability of diagnosing cause and manner of death during autopsy with the goal of increasing the validity of techniques, processes, and methods used in forensic medicine.

Use of opiate variants, including pain management medications such as morphine and street drugs such as heroin, have all been implicated in causing acute respiratory distress marked by non-cardiogenic pulmonary edema (NCPE). Despite efforts to treat patients who develop NCPE through chronic use or acute over-dose; presentation of NCPE stills has a mortality rate of 30-50%. With significant mortality and the rise in cases, development of NCPE is increasingly significant to the forensic community. The molecular and cellular mechanisms by which opiates induce non-cardiogenic pulmonary edema (NCPE) remain elusive. NCPE is a clinical hallmark of opiate use in long-term drug

users as well as patients treated with narcotics for chronic pain. Sporadic cases of NCPE were recently reported with use of other medications: primarily drugs used to treat other forms of edema, regulate blood volume, or blood pressure.

Although the pathogenesis of NCPE is largely unknown it thought to be dose related—thus maybe a presentation of an abhorrent cardiorespiratory response. Acute or chronic opiate use causes acute respiratory distress syndrome (ARDS) marked with pulmonary capillary leak and exudation leading to NCPE. Data shows us of opiates, primarily heroin, is the primary cause of NCPE in patients under 40. As many as 50% of these patients are clinically defined as an overdose with as much as 20% of these cases will be fatal. Previous animal models and marginal human studies identified three active opioid receptors ($\delta\mu\kappa$) varying in distribution throughout the respiratory tract. The lung is a very complicated microenvironment. Several hypotheses regarding the pathogenesis of NCPE indicate involvement of various cell and tissue types throughout the respiratory tract. Local changes may cause alterations to the alveolar epithelium direct or have effects on the pulmonary capillary bed resulting in NCPE. The lung parenchyma co-exists with the alveolar terminal air space where gas exchange occurs. Studies indicate there are two distinct $H^3$-morphine binding sites—with the most abundant binding localized within alveolar walls. Therefore, this is the site implicated as responsible for fluid clearance in the lungs. The exact mechanism by which activation of opiate receptors in this region leads to fluid influx is largely unknown. It is possible alveolar tissue plays a role in the release of soluble mediators or recruitment of inflammatory cells leading to a cascade of events contributing to the pathogenesis of NCPE. Dysregulation of solute and fluid clearance by the alveolar epithelium itself may be altered by opiate receptor activation. Finally, long-term or acute aactivation of opiate receptors with may lead to significant alterations in the epithelial surface that are the basis local changes conducive to the onset of NCPE.

With underlying disease in the lungs or other chronic conditions which require use of opiates, these changes may not be easily recognizable during autopsy. The goal of this poster is to demonstrate how opiate toxicology may induce local effects in the respiratory tract which ultimately results in direct changes to the pulmonary alveolar epithelium contributing to underlying disease. Secondary pathology may contribute to cause and manner of death in forensic cases. Understanding how opiates contribute to altered pathology will enhance the methods by which forensic pathologists diagnose NCPE postmortem.
**Autopsy, Forensic Pathology, Pulmonary Toxicology**

## G16 Is Toxicological Analysis Necessary in Postmortem External Examinations?

*D. Kimberley Molina, MD\*, Bexar County, Medical Examiner's Office, 7337 Louis Pasteur Drive, San Antonio, TX 78229; and Meredith A. Lann, MD\*, UCDHSC, AIP - Department of Pathology, 12605 East 16th Avenue, Room 3026, Aurora, CO 80045*

After attending the presentation, the attendee will understand the decision process of performing an external examination versus a complete or partial autopsy in medicolegal cases and the potential ramifications of not ordering a full toxicologic panel on cases where a postmortem external examination was performed.

The presentation will impact the forensic community by serving as a critical part in the decision making process of medical examiners in deciding how to analyze medicolegal cases and will serve to augment the literature used to establish the standard of practice for the performance of external examinations.

In many jurisdictions, external examinations are performed rather than complete autopsy examinations in certain types of medicolegal cases. Deaths in elderly patients or deaths after a fall are just a couple examples which may be included in such cases. In many of these cases,

the cause of death appears to be readily apparent from the medical records and/ or circumstances of death and toxicology is not performed.

A retrospective review of all external examinations performed at the Bexar County Medical Examiner's Office during a five year period (2003 - 2007) was undertaken comparing cases in which: toxicology was not performed; toxicology was performed but did not alter the cause and manner of death; and toxicology was performed and altered the cause and manner of death.

It was found that in cases where toxicology was performed, the toxicology results altered the cause and/ or manner of death in an average of 1.8% of cases. If toxicology had been routinely ordered on all external examinations at the BCMEO, it would have theoretically altered approximately 21 additional cases during the five- year period.
**Toxicology, External Examination, Cause and Manner of Death**

## G17 TASER® Wound Progression in Two Deployment Modes

*Donald M. Dawes, MD\*, Lompoc Valley Medical Center, 518 East Hickory Avenue, Lompoc, CA 93436; and Jeffrey D. Ho, MD, Hennepin County Medical Center, 701 Park Avenue South, Department of Emergency Medicine, Minneapolis, MN 55415*

After attending this presentation, attendees will have a better understanding of the wounds created by the TASER® X26 device.

This presentation will impact the forensic community by assisting in the identification of characteristic wound patterns created by the TASER® X26, the most commonly used conducted electrical weapon.

**Introduction:** Conducted electrical weapons are used by law enforcement to control violently resistive subjects. The TASER® X26 is the most commonly used conducted electrical weapon. It can be used in the probe-deployment mode in which probes are fired from the device at the subject, or it can be used in the drive-stun deployment mode in which the device is physically touched to the subject. The two deployment strategies can create different signature wound marks. To date, there is no study that has attempted to catalogue and describe these marks.

**Methods:** Subjects were recruited from police training classes for the study. The subjects were to receive an exposure from a TASER X26 as part of their training class. Subjects were allowed to choose between the two deployment modes depending on the rules of their class. Subjects completed a screening questionnaire that included the Fitzpatrick scale. The exposures were five seconds or less. Subjects had photographs of the wounds taken after the exposure immediately, and at 24, 48, and 72 hours, as well as at one month.

**Results:** The two deployment strategies left differing marks. The probe deployment mode generally created circular superficial partial thickness burns. The drive-stun mode created variable marks depending on the movement of the subject which included irregular superficial partial thickness burns that may be paired at about 40 mm (the distance between the metal contact points on the device), but not necessarily so. This mode also created abrasions and contusions. Some subjects had persistent hyperpigmented marks at one month.

**Conclusions:** The two probe deployment modes left different marks. It may be important for forensic examiners to be able to distinguish these marks from other minor skin trauma. Studies such as these will help in this.
**TASER®, Wound, Burns**

## G18 Rat Bite Fever: A Fatal Case of *Streptobacillus moniliformis* Infection in a 14-Month-Old Boy

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After attending this presentation, attendees will understand the features of *Streptobacillus moniliformis*, are, under recognized infection as they apply to a forensic setting through an autopsy case presentation.

This presentation will impact the forensic sciences community by highlighting the key features of *S. moniliformis* infection including the clinical presentation, postmortem diagnosis of *S. moniliformis* infection, and risk factors as they relate to a particular forensic autopsy case.

After viewing this presentation attendees will understand the features of *S. moniliformis*, a rare, under recognized infection as they apply to a forensic setting through an autopsy case presentation.

Rat Bite Fever, caused by *S. moniliformis* infection, is an acute syndrome of fever, rash, and migratory polyarthritis. In the United States, primarily children under the age of 12 years are infected with a total of less than 200 cases reported. Common vectors include rats and mice, which are natural reservoirs. Transmission is predominantly from a bite or scratch, but contact with or ingestion of food contaminated with feces or saliva has also been reported.

A previously healthy 14-month-boy died after a rapid decline after onset of fever and a diffuse rash over his face, trunk, and extremities. Crime scene investigation revealed a disheveled, cluttered bedroom where the child's crib was located. Several markedly soiled animal cages were adjacent to the crib containing rabbits and ferrets. The room was also infested with roaches, flies, and ticks over the floor, walls, ceilings, and all of the bedding. A complete autopsy, including laboratory testing, revealed a well-developed and well-nourished white male infant with normal age-adjusted height and weight. A red-pink macular and mostly confluent rash covered almost the entire body surface with prominence on the head including the scalp, neck, anterior and posterior torso, anogenital region, and portions of the thighs without mucosal involvement. There was sparing of the bilateral legs, soles, palms and portions of the forearms, nose and mouth, except the left lateral corner of the mouth. The rash did not involve the buccal mucosa or gums. The right knee had a donut-shaped bite rash suspicious for a bite mark. Internal examination revealed a mildly enlarged, congested liver and enlarged mesenteric lymph nodes. Microscopic examination of the lungs showed interstitial pneumonitis with rare neutrophils and edema. There were focal areas of gastric aspiration without associated vital reaction. The kidneys had fibrin micro-thrombi with focal fibrinoid necrosis of the tubules, consistent with Disseminated Intravascular Coagulopathy. Microbiologic culture of cerebrospinal fluid was positive for *S. moniliformis* while routine blood cultures were negative. Viral cultures were also negative. Routine toxicologic analysis of heart blood and liver revealed diphenhydramine administered during resuscitation.

In the United States, 55% of cases of Rat Bite Fever occur in children less than 12 years of age. The demographics of the victims have broadened to include children, pet store workers, and laboratory technicians, because the rats have become popular pets and study animals. The infection is associated with a mortality rate of 7-13%, if untreated. The actual rate of infection may be much higher, because it is not a reportable disease. Although easily treatable with antibiotics, the diagnosis and treatment can be delayed due to a broad differential diagnosis which includes meningococcemia, *Staphylococcus aureus* or *Streptococcus pyogenes* septicemia, Rocky Mountain Spotted Fever, or other Rickettsial diseases, enterovirus infection, disseminated gonorrhea, Lyme disease, ehrlichiosis, brucellosis, leptospirosis, and secondary syphilis. Given this differential of more common entities, laboratory identification is essential to proper diagnosis. This paper shows the importance of considering *S. moniliformis* as an etiology

In all suspected cases, a complete autopsy should be performed and the microbiology lab should be contacted for guidance in submitting blood, cerebrospinal fluid, and probably synovial fluid in appropriate media supplemented with 20% blood serum or ascitic fluid to prevent growth inhibition of *S. moniliformis*.

**Rat Bite Fever, Streptobacillis moniliformis, Actinobacillus muris**

## G19 Contributions From Forensic Imaging to the Investigation of Fatal Upper Cervical Fractures

*Lars Uhrenholt, PhD, and Lene W. Boel, PhD\*, University of Aarhus, Institute of Forensic Medicine, Brendstrupgaardsvej 100, Aarhus N, 8200, DENMARK*

After attending this presentation, attendees will understand the value of advanced diagnostic imaging procedures in forensic medical investigations of upper cervical spine fractures following trauma.

This presentation will impact the forensic community by showing how upper cervical spine fractures are frequently seen in relation to fatal trauma to the head and neck, and where this anatomical region may be difficult to evaluate during medicolegal autopsy, the contributions from advanced diagnostic imaging procedures may be of great importance to the investigation.

The purpose of this presentation is to present the value of advanced diagnostic imaging procedures in the forensic medical investigations of upper cervical spine fractures following trauma.

Upper cervical spine fractures are frequently seen in relation to fatal trauma to the head and neck and, where this anatomical region may be difficult to evaluate during medicolegal autopsy, the contributions from advanced diagnostic imaging procedures may be of great importance to the investigation.

The upper cervical spine is clinically a very important anatomical region, where the high degree of mobility is obtained on the expense of poor stability. Several types of fractures are possible at the atlas (C1) and axis (C2) vertebrae. Five cases have been retrieved where different types of trauma, (e.g., road traffic crash collisions, fall, blow to the head from moving objects), had occurred causing fractures to the upper cervical vertebrae. Each of the deceased was examined using advanced computed tomography, an in-house Siemens Definition 64 slice dual-energy scanner facility, as adjunct to the medicolegal autopsy. The upper cervical spine was reconstructed using sub-millimeter slice thicknesses and all images were examined in three planes (horizontal, coronal, and axial) as well as using 3-dimensional reconstructions. The findings from the CT-scanning were correlated with the findings from the medicolegal autopsy and the contributions from the forensic imaging procedures to the medicolegal investigations were evaluated.

The review of five unique cases with upper cervical spine fractures showed that forensic imaging procedures in combination with medicolegal autopsy allow very detailed evaluation and categorization of fractures. Although fractures of the odontoid process were readily identified during autopsy, the exact classification according to the system by Anderson and D'Alonzo was made possible by examination of the CT-images. The fractures of the atlas were more difficult to visualize during the medicolegal autopsy, particularly at the posterior arch, whereas the diagnostic imaging procedures allowed clear identification as well as classification of the fractures according to the system proposed by Jefferson.

This presentation of five trauma cases showed that advanced diagnostic imaging procedures contributes significantly to the forensic medical investigations of upper cervical spine fractures following trauma. This is important as implementation of such adjunct procedures

to the medicolegal autopsy may strengthen the degree of detail of the investigation. Although this is a small group of selected trauma cases, this presentation highlights some of the major advantages achieved by expanding the forensic investigations to also include forensic imaging procedures.

A number of cases that have in common the presence of upper cervical spine fractures will be discussed. The contributions to the medicolegal investigations from advanced computed-tomography scanning will be presented and it is recommended that forensic specialists become familiar with the potential of advanced imaging procedures to the medicolegal investigations.

**Cervical Fracture, Forensic Imaging, Postmortem Autopsy**

## G20    Ankylosing Spondylitis in Traumatic Death: A Case Report

*Asser H. Thomsen, MD\*, Lars Uhrenholt, PhD, and Annie Vesterby, MD, DMSc, Institute of Forensic Medicine, University of Aarhus, Brendstrupgaardsvej 100, Aarhus N, DK-8000, DENMARK*

After attending this presentation, attendees will have a better understanding of the possible impact of pre-existing structural skeletal disease in traumatic death, illustrated by a case report.

This presentation will impact the forensic community by reminding it of the importance of taking all natural disease into account when investigating deaths, even those diseases not normally considered fatal. Furthermore, it will present the possibilities of advanced radiological imaging as a facilitator in death investigation in the evaluation of the mechanism and manner of death.

Ankylosing spondylitis is a rheumatic disease which is associated with tissue type HLA-B27 and is considered non-fatal. Main structural features in severely affected individuals are osseous fusion of the sacroiliac joints, and rigidity of the spinal column caused by bone bridging between vertebral bodies (syndesmophyte formation). Due to this rigidity there is an increased risk of spinal fractures, especially cervical fractures, even from low energy trauma.

A middle-aged man rode his bike home from a bar while intoxicated. A witness saw him swaying and at low speed riding the bike into a curbstone. During the crash he went over the handlebars and collided with the pavement face first. His breathing ceased immediately, soon followed by cardiac arrest. Resuscitation efforts at the scene were unsuccessful. Due to the rapidity of the cardiac arrest, the attending emergency physician ruled that it was a natural death caused by a cardiovascular event secondary to the fall from the bike. According to the antemortem information obtained from the police report and the general practitioner, the deceased was healthy without prior cardiovascular disease. Postmortem computed tomography scanning revealed multiple fractures of the spine, including a fracture of the odontoid process of C2, disco-vertebral avulsion through C3-C4, and Th10-Th11. Associated with the upper cervical fractures there was displacement of fragments into the spinal canal affecting the spinal cord. Furthermore, there were ankylosing changes of the anterior longitudinal ligament throughout the spinal column with extensive syndesmophyte formation bilaterally, also known as bamboo spine configuration, particularly in the lumbar spine. The sacroiliac joints were closed by osseous fusion. All the radiological findings were in agreement with the diagnosis of ankylosing spondylitis. The medicolegal autopsy showed abrasions in the face, on the back, on hands, and legs; bleeding in and around the spinal fractures; rib fractures with sparse bleeding; bone bridging of the intervertebral joints; an enlarged heart, insignificant atherosclerosis; a fatty liver, and an enlarged spleen. Blood alcohol was 189 mg/dl. The microscopical examination revealed hypoxic changes in the brain, granuloma formation in the lungs consistent with sarcoidosis, and bone marrow emboli in the pulmonary arteries. The bone marrow emboli were thought to come from the primary spinal fractures or the secondary rib fractures caused by the resuscitation efforts.

Further investigation into the medical history, by requesting relevant hospital records, revealed that the deceased had received treatment in an outpatient clinic nine years prior, due to ankylosing spondylitis with rigidity of the spine. He had furthermore been under evaluation for lung sarcoidosis. The cause of death was ruled to be upper cervical spinal cord injury due to upper cervical spine fractures, complicated by spinal rigidity secondary to ankylosing spondylitis. The manner of death was ruled to be accidental.

This case report illustrates the fatal outcome of spinal injuries in an individual who suffered from a structural skeletal disease, where the ankylosing spondylitic changes acted as predisposing factors leading to his death. Thus, knowledge of pre-existing skeletal disease is important in the medicolegal evaluation, as diseases that are considered non-fatal can contribute to the cause of death. In this case report the understanding of the conditions leading to death was recognized and supported by the postmortem computed tomography.

This presentation will impact the forensic community by reminding it of the importance of taking all natural disease into account when investigating deaths, even those that are not normally considered fatal. Furthermore, it will present the possibilities of advanced radiological imaging as a facilitator in death investigation in the evaluation of the mechanism and manner of death.

**Ankylosing Spondylitis, Postmortem Examination, Forensic Imaging**

## G21    An Unsolved Cold Case in Iowa: A Probable Case of Dragging

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After attending this presentation, attendees will learn about differential diagnosis for trauma due to dragging. Dragging injuries to human remains is rarely described in the literature and is limited to accidental long-range towing behind large vehicles.

This presentation will impact the forensic community by describing the skeletal morphological changes associated with dragging from a different context than is currently found in the literature.

Dragging injuries to human remains is rarely described in the literature and is limited to accidental long-range towing behind large vehicles. The goal of this presentation is to provide a differential diagnosis for trauma due to dragging. This presentation will impact the forensic community by describing the skeletal morphological changes associated with dragging from a different context than is currently found in the literature.

On October 4, 1978, decomposed human remains were found lying face down in a ditch in a rural portion of Northwestern Iowa. The female victim was partially clad in knee-high white "go-go" boots with her panties and pants bunched up under the torso. Her arms were stretched over her head and her ankles were tied together with a knotted rope. An autopsy the following day revealed no obvious traumatic injuries to the soft tissue or skeleton other than damage to the maxilla that was originally attributed to animal gnawing. The hands were retained and the skull, clavicles, pubic symphyses and possibly other bones were sent to a forensic anthropologist for analysis. Ultimately the identity of the victim and the cause of death were unknown and the case became "cold."

Interest in the case was renewed nearly two decades later when, in January 2006, the victim's fingerprints were matched to those on a

fingerprint card of a woman arrested in California in 1973. Mitochondrial DNA tests of metacarpal bones of the victim positively matched the mtDNA of a known daughter of the California woman. The victim was identified as a 23-year old prostitute from California who was last seen in Georgia in February of 1978. Throughout 2006 investigators created a list suspects, including the victims' ex-husband, but most of these individuals were deceased or could not be found for questioning. In 2007 the remains were exhumed, the bones that had been sent to other anthropologists were returned, and a new autopsy was ordered to further investigate the cause of death.

The soft tissue of the dorsal aspect of the entire body was remarkably well preserved while the ventral aspect (which had been in contact with the ground) was skeletonized. A comprehensive drug panel on decomposed skeletal muscle was positive only for caffeine and cotinine. Following the forensic pathological examination the bones were macerated in warm water with detergent and examined by the anthropologist. Bone loss of the maxillary alveolar bone, hard palate, anterior nasal spine, and nasal aperture was extensive. Adherent bone fragments, radiating fractures, tool marks, or animal gnawing were absent and the morphology was most consistent with abrasion. Similarly, abrasion injuries were apparent on the medial aspects of both elbows (distal humeri and proximal ulnae) and the anterior iliac spine of the right ilium. No bony modifications were observed below the pelvis.

The abrasion injuries are consistent with dragging in a prone position with the arms over the head as the lower face, medial elbows and one or both ilia would be in contact with the ground. The mandible and anterior rib cage are also expected to be affected but unfortunately these elements were not retained from the original autopsy. Only two known cases of dragging are published in the literature and these involve dragging behind or under a vehicle for significant distances (at least 2.5 miles). One case (Klintschar et al. 2003) reports a body dragged prone by one foot with the arms over the head such that the medial aspect of the elbows faced outwards. The medial humeri and ulnae as well as the lower teeth were extensively abraded. In the current case the injury pattern, the position of the pants (pulled up under the torso), the position of the ligature around the ankles and the final resting position of the body in the ditch suggest the victim was pulled by the feet while in a prone position such that the legs were off the ground and the arms over the head. The remote location and terrain implies the victim may have been dragged by a vehicle down a gravel road but most likely was pulled by hand. While coffin abrasion cannot be completely ruled out, these particular bones were not observed to be in direct contact with the metal sides when the coffin was unsealed. Thus, a mechanism of manual dragging is proposed to explain the morphology and distribution of the skeletal injuries.

**Trauma, Forensic Pathology, Forensic Anthropology**

# G22 An Unusual Death Involving a Sensory Deprivation Tank

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After attending this presentation, attendees will understand the intended use of sensory deprivation tanks and understand possible risks associated with improper use. A practical investigative approach to similar deaths involving water tanks and spas will be discussed.

This presentation will impact the forensic community by familiarizing the forensic community about the use of sensory deprivation or flotation tanks, as well as risks associated with improper use.

Deaths involving sensory deprivation tanks, also called flotation tanks, are very rare. A thorough literature search using PubMed and

Ovid MEDLINE search engines yielded no such cases; however, recently a preliminary report on a similar case from Berkshire, UK was reported online in late 2007. This is the first known death associated with a flotation tank to be reported in the medical literature.

Flotation REST (Reduced Environmental Stimulation Therapy) is used by some as a modality for stress-reduction or for behavioral modification programs. REST was initially a research tool for neuropsychiatric studies in the 1960s, but became more popular in the United States in 1970s-1980s when the tanks became available for commercial and personal use. Most recently some medical practitioners ascribe to its use as an alternative therapy for various medical illnesses, as it may reduce hypertension and alleviate chronic pain. The medical literature discusses the effects of chamber REST for many psychiatric, behavioral and addiction disorders; however controlled studies using flotation REST are very limited.

A unique case in which a previously healthy 50-year-old woman apparently died while floating in a sensory deprivation tank within the basement of her own home will be described. The deceased reportedly had not previously used the tank, although had purchased it approximately three years previously, and was likely not familiar with the proper use of the tank. At the time of the scene investigation the unit's filtration system, which was situated close to the flotation tank, was noted to be on and running. The temperature of the water in the flotation tank was elevated at 116 deg F, approximately 20 deg F higher than the usual target temperature for flotation sessions. Examination of the tank and accessories found all components operating within specifications, with no malfunctions or electrical hazards identified. There was no evidence the decedent drowned, as the nose and mouth were not submerged. A full medicolegal autopsy was performed. No anatomic cause of death was identified at autopsy. Postmortem laboratory studies demonstrated a vitreous creatinine of 5.2 mg/dl, a blood ethanol level of 0.270%, an elevated blood doxylamine level, and the presence of sertraline and diphenhydramine. It has been concluded that the deceased inadvertently left the pump on during her flotation session, which resulted in the elevation of water temperature after she fell asleep during the session. The cause of death was determined as due to acute mixed drug and ethanol toxicity with probable hyperthermia contributing. Manner was ruled as accident.

It is recommended that mind-altering or CNS depressant drugs including alcohol not be used during flotation REST sessions. This case report and discussion will help the forensic community understand the use of flotation tanks and the risks associated with improper use. Investigation of deaths involving these unit or similar devices such as bathtubs or spas should include special precautions aimed to prevent harm to the investigation crew. In addition, full examination of the tank and accessories should be performed by professionals familiar with the equipment to confirm any product malfunctions or other potential safety hazards.

**Sensory Deprivation Tank, Flotation Tank, Intoxication**

# G23 Preliminary Analyses of Carrion Colonization of Necrophagous Flies (Diptera: Calliphoridae) in Central Oklahoma

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After attending this presentation, attendees will have a better understanding of the colonization of carrion by necrophagous flies in two suburban habitats in central Oklahoma. In addition, attendees will

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better understand factors that impact necrophagous fly colonization of carrion and their diversity suburban habitat.

From this presentation, the forensic community will attain a better appreciation for carrion insects in central Oklahoma and environmental conditions for postmortem interval (PMI) estimations of human remains based on associated arthropod fauna.

This presentation will impact the forensic community by demonstrating the potential influence of environmental factors on carrion colonization by necrophagous flies and vertebrate carcass recycling in non-vegetated habitats in central Oklahoma, and the importance of insular suburban woodlots as a refuge and species pool for dipteran decomposers. With the possible onset of global warming and the continuing expansion of human habitation, maintaining suburban woodlots and vegetated green zones may prove critical for the preservation of terrestrial decomposer populations and other wildlife .

Necrophagous flies are important ecologically and forensically. Ecologically, carrion frequenting flies (Diptera: Calliphoridae) are dominant members of the terrestrial decomposer community and, as such, play a significant role in the recycling of vertebrate remains, improving public health. Additionally, analyses of immature and adult flies colonizing remains can provide a broad spectrum of forensically meaningful information, including estimates of the minimum postmortem interval (PMI). Central to an understanding of the ecological and forensic significance of necrophagous Diptera is knowledge of the environmental factors potentially limiting carrion detection, access, and colonization. This study examined the impact of high temperatures and surface winds on the colonization of carrion (liver) by necrophagous Diptera in two suburban Oklahoma habitats. Standardized samples of beef liver (uniformed attractant) were placed in a suburban woodlot and turf grass field in Central Oklahoma. Wind speed, wind direction, and temperature were measured at each site and correlated with carrion fly colonization rates and species diversity. Over 100 replicates were conducted over the course of 8 weeks. Fly colonization patterns were compared with commercial flytrap (Pherotech ®) and rodent carcass trials.

Study results indicated a clear difference between habitats, with the turf field characterized by stronger winds, higher temperatures, more rapid carrion desiccation, reduced fly colonization rates, and lower species abundance and diversity. Additionally, the turf field habitat was characterized by a significantly greater number of days devoid of carcass colonization by carrion flies. Vegetative stratification, characteristic of the suburban woodlot habitat, provided mediation of wind and heat effects and facilitated increased carrion fly abundance, diversity, and activity.

The study demonstrates the potential influence of environmental factors on carrion colonization by necrophagous flies and vertebrate carcass recycling in non-vegetated habitats in central Oklahoma, and the importance of insular suburban woodlots as a refuge and species pool for dipteran decomposers. With the possible onset of global warming and the continuing expansion of human habitation, maintaining suburban woodlots and vegetated green zones may prove critical to the preservation of terrestrial decomposer populations and other wildlife .

**Forensic Entomology, Carrion Colonization, Necrophagous Diptera**

## G24 Two Fatal Cases of Hidden Pneumonia in Young People

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The goal of this presentation is to review two fatal cases of hidden pneumonia in young people.

This presentation will impact the forensic science community by demonstrating how acute respiratory distress syndrome (ARDS) can result in death. Also young people generally, in cases of pneumonia, can be treated and consequently prevent death. Therefore, these cases illustrate the importance of early diagnosis of this condition.

ARDS is a severe lung disease characterized by inflammation of the lung parenchyma leading to impaired gas exchange with concomitant systemic release of cytokines and inflammatory mediators frequently resulting in multiple organ failure (MOF). This condition usually requires a rapid application of mechanical ventilation and admission to an intensive care unit.

When the endothelium of lung capillaries and the alveolar epithelium are damaged, plasma and blood spread in the interstitial and intralveolar spaces. Such a change induces decreased lung compliance, pulmonary hypertension, reduced functional capacity, modified ventilation/perfusion ratio, and hypoxemia. ARDS can occur within 24 to 48 hours of an attack of acute illness. In such a case the patient usually has shortness of breath and tachypnea.

Typical histological presentation involves diffuse alveolar damage (DAD) and hyaline membrane formation in alveolar walls.

If the underlying disease is not diagnosed and treated, the condition of the patient will worsen resulting in shock and/or MOF potentially resulting in sepsis.

Supposedly over 30% of ARDS cases are due to "sepsis syndrome," which is characterized by leukocytosis or leukopenia, fever, hypotension and leading to the identification of a potential source of systemic infection via positive blood culture for pathogenic agents.

The rate of survival in case of severe ARDS with appropriate and early treatment is 50%. However, if the severe ARDS induced hypoxemia is not recognized or treated, or if the disease reaches is not diagnosed until the terminal phase, cardio-respiratory arrest occurs in more than 90% of patients.

**Case 1:** A 29-year-old man was found lifeless at home by his girlfriend. Death scene investigation was unremarkable. He took psychotropic drugs, and he was known to be an abuser of alcohol and drugs. Family history was negative for sudden death. A complete postmortem examination was performed four days after death. External examination was insignificant. The internal examination revealed polyvisceral congestion, microthrombosis, cerebral and pulmonary oedema. Free citrine liquid was found on both sides of the pleural cavities.

Marked congestion and release of foamy material on sectioning of both lungs was observed. Hydrostatic docimasia for large and small fragments was positive in all fields such as an index of bilateral consolidation. The histological lung examination, performed with routine haematoxylin-eosin staining, revealed diffuse alveolar damage, endobronchial and endoalveolar infiltrates of polymorphonuclear neutrophilic leukocytes and focal emphysema. No fungal infections

were detected using slides by PAS and Grocott staining. Gram staining didn't reveal evidence of bacteria. Toxicology was negative for drugs and alcohol.

**Case #2:** A 31-year-old man was with a history of pharingodinia, fever, and cough taken to the hospital. The clinical symptoms progressed to acute onset of increasing shortness of breath rapidly progressing to acute respiratory failure with haemoptysis. Chest x-ray demonstrated bilateral diffuse airspace opacification; the high resolution CT confirmed the presence of bilateral diffuse airspace consolidation associated with liquid in pleural cavities. The patient, with a severe leukopenia, was admitted to the intensive care unit, but died after a few hours. Two blood cultures were positive for group A beta-hemolytic *Streptococcus*. No other pathogenic agents were present. An autopsy was performed within 48 hours. The internal examination revealed an increase in lung weight and findings were consistent with intense congestion attributable to a bilateral pneumonia. The histological examination of lung specimens showed a pattern of diffuse alveolar damage and the presence of intralveolar bacterial and fungal colonies. In the kidneys a thrombotic microangiopathy compatible with DIC was found.

In conclusion, the cause of death was, in both cases an acute cardio-respiratory failure secondary to acute bilateral pneumonia with DAD and consequently ARDS, sepsis and DIC.

**Hidden Pneumonia, Diffuse Alveolar Damage, Adult Respiratory Distress Syndrome**

# G25    Methodologies for Heteroplasmy Identification

*Simona Ricci, MD\*, Department of Legal Medicine, Viale Regina Elena 336, Roma, ITALY*

After attending this presentation, attendees will receive information about the detection and study of heteroplasmy in the forensic field.

This presentation will impact the forensic community because it shows two caseworks in which different techniques were implemented to achieve good results.

Mitocondrial DNA (mtDNA) sequencing has been considered a useful tool for forensic analysis, and it is typed routinely in forensic analyses to assist in determining the source of old bones, teeth, hair shafts, and other biological samples where nuclear DNA content is too low or degraded to genotype by analyzing autosomal short tandem repeat (STR) loci.

Typically, forensic mtDNA data are obtained by sequencing (i.e. Sanger method, followed by electrophoresis and fluorescent detection) the two hypervariable regions (HV1 and HV2) of the noncoding control region of the human mtDNA genome. Traditionally, sequencing has been the method of choice because all polymorphisms contained within the amplified fragment can be detected. The definition of heteroplasmy is the existence of two types of mtDNA within an individual. It is known that the sensitivity of heteroplasmy detection is method-dependent, and the most fundamental approach to sampling the individual mtDNA present in an individual is achieved through cloning.

The most common form of heteroplasmy observed in the mtDNA control region is length heteroplasmy. Depending on its extent, length heteroplasmy may result in an inability to read or interpret sequence data and must be compensated for with alternative sequencing strategies. There are different methods to evaluate the mutation load of defective mtDNAs: primer extension, TTGE, RT-PCR, restriction fragment analysis and SSCP. In addition heteroplasmy using SSO typing, DHPLC/nuclear loci and DGGE can be detected. These methods are highly sensitive and can detect and sometimes quantitate heteroplasmy at levels lower than 1%. In this study, information regarding the management of different cases using clonage and DHPLC respectively will be presented. In both cases heteroplasmies were present. The aim of this work is to demonstrate the utility of these two techniques showing the main indications and advantages for the forensic community.

**mtDNA, Heteroplasmy, Clonage vs. DHPLC**

# G26    Internal Validation of Quantifiler™ DUO DNA Quantification Kit and AmpFℓSTR® Yfiler™ PCR Amplification Kit

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After attending this presentation, attendees will learn about the methods and results from the validation study of two Y specific kits performed at the Institute of Forensic Science of Puerto Rico.

This presentation will impact the forensic community by communicating the methods and results from a validation study of two available forensic kits. Y-locus specific kits are an important forensic tool to aid in the discrimination of the male contribution in a sample, such as rape cases.

The DNA-Serology Laboratory of the Institute of Forensic Sciences of Puerto Rico is the only Latin American laboratory accredited by ASCLD-LAB. Y-STR's have become an important forensic tool in cases in which male-male or male-female mixtures arise, such as rape cases. Y-locus STR's have also gained importance in the clarification of erroneous Amelogenin tests from autosomal STR amplification kits. Quantitation of human male DNA and Y-STR analysis are not currently performed in-house. Therefore, this study was designed to validate two commercially available forensic kits, Quantifiler® Duo DNA Quantification, and AmpFℓSTR® Yfiler™ PCR Amplification for in-house quantitation of human male DNA and Y-STR detection. These kits were validated for use with one real-time PCR instrument model for DNA quantitation (ABI Prism® 7500 Sequence Detection System) and two capillary electrophoresis instrument models (ABI Prism® 3130*xl* and 3100-*Avant* Genetic Analyzers) for Y-STR detection.

Quantifiler® Duo DNA Quantification Kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in one reaction. The quantification assay combines three 5' nuclease assays, namely: target-specific human DNA assay, target-specific human male DNA assay, plus an internal PCR control (IPC). The human target is Ribonuclease P RNA Component H1 (RPPH1) located at 14q11.2 and 140 bases long is detected by TaqMan® MGB probe labeled with VIC dye. The male target is the sex-determining region Y (SRY) located at Yp11.3 and 130 bases long is detected by TaqMan® MGB probe labeled with FAM dye. The internal PCR control is a synthetic sequence not found in nature. It is 130 bases long and is detected by TaqMan® MGB probe labeled with NED dye. AmpFℓSTR® Yfiler™ PCR amplification kit is a short tandem repeat multiplex for human male-specific DNA amplification that includes the European minimal haplotype loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, and DYS 393), the SWGDAM recommended loci (DYS 438 and DYS439), and additional highly polymorphic loci (DYS437, DYS448, DYS456, DYS458, DYS635, and Y GATA H4) for a total of 17 Y-STR loci in a single PCR reaction.

Internal validation studies included: precision, accuracy, sensitivity, male:male mixture evaluation, female:male mixture evaluation, stutter determination for each locus, as well as forensic casework. Forensic

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casework includes a double-rape case, an incest case, a proficiency test from Collaborative Testing Services, Inc., and a case with null Amelogenin typed with AmpFℓSTR® Identifiler™ PCR Amplification kit. In addition to these studies, 106 samples were used to create a Y-STR database for Puerto Rico. All validation study data is being analyzed and will be discussed.

Internal validation of available DNA typing kits for human identification allows for the evaluation of a procedure's efficiency, reliability, performance characteristics, and limitations. The internal validation of the Y-locus specific kits (AmpFℓSTR® Yfiler™ and Quantifiler® Duo) will allow the Puerto Rico Institute of Forensic Science DNA laboratory to save time and money, as well as the ability to offer reliable male DNA typing services.

**Y-STR, Male Quantitation, Yfiler™**

# G27 The Effect of Clothing on Scavenger Visits and Decomposition

*Amanda J. Marshall, BS\*, Jennifer R. Simon, BS\*, and Phillip L. Watson, PhD\*, Ferris State University, Department of Biology, 2004 ASC, 808 Campus Drive, Big Rapids, MI 49307*

After attending this presentation, attendees will learn the differences clothing can make on the timing and frequency of scavenger visits to remains. Clothing will be shown to significantly affect when scavengers visit and alter the death event.

This presentation will impact the forensic community by demonstrating how time of death is determined by many factors, the presence or absence of clothing does affect the timing of visits.

The presence or absence of clothing can alter the decomposition rate (Anderson 2001, Kelly 2006). Quantifying the decomposition rate is difficult and complicated by the potential differences in timing of scavenger visits and alterations to the death scene by those visits. This study, which is a follow-up to one conducted in 2007, examined the rate of decomposition on a clothed and unclothed pig as a function of summer environmental conditions, but includes motion sensor cameras to capture scavengers frequenting the sites. Insects were collected twice a day until the dry remain stage occurred. Cameras were secured and pictures were obtained as motion was sensed by the camera. Temperature, relative humidity, rainfall, and wind speed data were collected on an hourly basis. The data show increased activity of forensically important insects as a function of temperature and clothing. The delay of the clothed victim to reach the dry remains stage was significantly different from the delay for the victim without clothing. The development stages of larvae collected from the clothed victim were also significantly smaller than the unclothed victim at all collection dates until the unclothed victim was no longer attractive to forensically important flies. The scavenger visits were significantly different between the two test animals in terms of time and abundance as determine by motion sensor cameras. How scavengers may be useful in determining state of decomposition will be discussed.

Data to be discussed will be the differences in larva size, insect species composition on each pig over time and identity, frequency and timing of scavenger visits. Comparisons were done as an ANOVA test and a species diversity comparison for all days. Results will be used to set up teaching mock crime scenes to illustrate the effects of clothing on PMI calculations.

**Scavengers, Clothing, Insects**

# G28 Foreign Bodies: Three Cases of Projectile Maintenance Without Complications

*Duarte N.P. Vieira, PhD\*, Carlos Abreu, MD, and Cristina Cordeiro, MSci, Instituto Nacional de Medicina Legal, Largo da Sé Nova, Coimbra, LB 3000-213, PORTUGAL*

After attending this presentation, attendees will learn about the possibility of retention of foreign bodies, like projectiles, in the body during long periods of time without any symptoms or evident complications, and that are only accidentally discovered during an autopsy performed due to another cause of death. However, there are some cases where problems may occur, being the bullets the cause of several symptoms and even sudden death. Thus, a regular follow up of the victim must be done in these situations and sometimes a surgical removal is needed.

This presentation will impact the forensic community by showing three cases where projectiles were maintained in the body for several years, due to previous gunshot injuries. In one of these cases, the projectile was found during the autopsy of a traffic accident victim. Surprisingly it had remained in the pericardial cavity for 12 years! In the other two cases, concerning living persons, the projectiles stayed in the corpse of a dorsal vertebra and in the sphenoid sinus, respectively, for many years.

The possible complications and risks of bullets maintenance in victim's bodies are reviewed and the authors stress the particular attention that these situations demand during the medical forensic assessment of these individuals.

The retention of projectiles in the body is an unusual situation. Sometimes these projectiles are maintained for life in the body without any symptoms or evident complications, and are only accidentally discovered during an autopsy performed due to another cause of death.

**Foreign Bodies, Projectiles, Autopsy**

# G29 The Persistence of an Elevated Concentration of Ninhydrin-Reactive Nitrogen in Grave Soil

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After attending this presentation, attendees will understand that the concentration of ninhydrin-reactive nitrogen (NRN) in grave soil increases during the first year of decomposition while grave soil pH decreases.

This presentation will impact the forensic community by serving as a fundamental investigation into the estimation of extended PMI.

As time progresses, however, NRN concentrations fluctuate less making it difficult to estimate postmortem interval (PMI) over an extended period of time. This presentation will impact the forensic community by serving as a fundamental investigation into the estimation of extended PMI. Accurate estimates of extended PMI are currently difficult to achieve.

Although several methods to estimate postmortem interval (PMI) have been developed, there is no one method that can be used in all scenarios. Most of these methods focus on estimating early PMI (<30 days). As a consequence, relatively few methods have been developed to estimate extended PMI (>30 days). Recent investigations have shown that the decomposition of a body can have a significant effect on the chemistry of associated soil (grave soil) that persists into the extended

PMI. One effect is an increase in the concentration of materials (organic nitrogen and ammonium) that react with ninhydrin. This material is referred to here as ninhydrin-reactive nitrogen (NRN). Eventually, (NRN) concentrations will return to basal levels and it is believed that this can be used to estimate PMI. To determine how long NRN persists in soil, and develop a tool to estimate extended PMI, the NRN concentrations of grave soil associated with decomposing cadavers (after 0, 1 or 3 years) were measured.

The experimental site was located at the University of Nebraska Agricultural Research Development Center located approximately 48 km north of Lincoln, Nebraska, USA. The site is a pasture that is intermittently grazed by cattle and horses. The soil at the site is a deep silty clay loam of the Yutan series (Mollic Hapludalf). The climate is temperate midcontinental characterized by hot summers, cold winters, and moderately strong surface winds. Average annual precipitation is 695 mm. Approximately 75% of the precipitation occurs between April and September. Mean annual temperature is 9.8°C with mean minimum and maximum temperatures ranging from 0°C (January) to 31°C (July). The vegetation at site is dominated by non-native grass (smooth brougham) and forb (white clover) with some native vegetation, including daisy fleabane, yellowwood sorrel nut sedge, and pasture rose.

Swine (*Sus scrofa*) carcasses (~40 kg) plus a control (no cadaver) were used. Swine were killed with blunt force trauma to the cranium and placed on their right side on the soil surface facing west. Soil samples were collected (0-5 cm depth) from adjacent to the cadaver following 0 years, 1 year, and 3 years of decomposition and analyzed for NRN and pH. This experiment was replicated three times, which resulted in a total of six swine cadavers.

A significantly (P < 0.01) greater concentration of NRN was observed in grave soil after one year but not after three years. Also, a significantly (P < 0.01) lower pH was observed in grave soil after one year but not after three years. The current results demonstrate that the concentration of grave soil NRN and soil pH associated with a 40 kg cadaver can return to basal levels between one and three years postmortem. Thus, the maximum PMI that can be estimated using an increase in grave soil NRN or a decrease in grave soil pH is one year. Further research should be conducted to increase the accuracy of these approaches. In addition, other compounds and elements in grave soil should be investigated for their use in estimating PMI greater than one year.

**Forensic Taphonomy, Extended Postmortem Interval, Decomposition**

## G30     The Application of DNA Identification Technology to Large Wildlife Carnivore Attacks on Humans

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The goal of this presentation is to demonstrate the use of DNA typing to confirm the identity of wildlife predators in attacks on humans.

The growth and encroachment of human populations into rural areas, and the consequences of several years of drought, especially in the western United States, has resulted in more frequent contact with large predators such as mountain lions and bears, with sometimes fatal consequences for one or both. Correct identification of the species and individual animal is essential to ensuring public safety with minimal loss of endangered wildlife. The application of modern DNA technology to a fatal mountain lion and non-fatal mountain lion and a recent bear attack on humans with two-way and one-way transfers of DNA that enabled certain identification of the predator will be presented.

**Forensic DNA, STR, Puma**

## G31     Sudden Unexplained Death Due to Disseminated Malaria

*Ritesh G. Menezes, MD\*, Kasturba Medical College, Mangalore, India, Department of Forensic Medicine, Light House Hill Road, Mangalore, Karnataka 575001, INDIA*

After attending this presentation, attendees will understand that the demonstration of parasitized red blood cells with malarial pigment in the blood capillaries of internal organs by histopathology is a reliable and easy method of postmortem diagnosis of disseminated malaria.

This presentation will impact the forensic community by understanding the possibility of disseminated malaria as a cause of sudden unexplained death in malaria-endemic regions.

Sudden unexplained deaths are mainly attributed to the cardiovascular system and the respiratory system. A case study of sudden unexplained death due to disseminated malaria in an apparently healthy individual will be presented. In the present case, histopathological examination demonstrated the presence of parasitized red blood cells with malarial pigment in the blood capillaries in the brain, myocardium, pericardium, lungs, kidneys, liver, and the spleen.

**Sudden Death, Disseminated Malaria, Forensic Histopathology**

## G32     Chloride Levels of Sphenoid Sinus Fluid in Salt and Fresh Water Drownings on the Island of Oahu, Hawaii

*Nicole Stalter, MSFS\*, Chaminade University, 3140 Waialae Avenue, Forensic Science Department, Honolulu, HI 96816; Gayle Suzuki, MD, and Erin Mitsunaga, BA, Honolulu Medical Examiner, 835 Iwilei Road, Honolulu, HI 96817; and M. Lee Goff, PhD, Forensic Sciences Program, Chaminade University of Honolulu, 3140 Waialae Avenue, Honolulu, HI 96816-1578*

After attending this presentation, attendees will walk away with better knowledge of how chloride levels of sphenoid sinus fluid can help support pathologist's diagnosis of drowning as the cause of death.

This presentation will impact the forensic community by broadening research about chloride levels of sphenoid sinus fluid in salt and fresh water drownings.

There are many potential criteria to be evaluated in the pathologist's diagnosis of drowning as the cause of death. One of these involves the analysis of the sphenoid sinus fluid. In the drownings reported from the island of Oahu, Hawaii, chloride levels of sphenoid sinus fluid in salt water drownings are typically greater than 140 mmol/L and for fresh water drownings they are normally less than 65 mmol/L, however, to date there has been little research on the topic. During July 2007 through July 2008, there were 37 drownings reported on the island of Oahu. Nineteen of these had sphenoid sinus fluid removed and analyzed for the chloride content, 14 salt water and five fresh water. Eight of the 14 salt water cases had the expected chloride readings of greater than 140

mmol/L. Due to specific circumstances, including decomposition, the other six presented different results. Of the five fresh water cases, two presented the expected chloride levels of less than 65 mmol/L, while three had concentrations greater than 65 mmol/L. Of these, two were recovered from chlorinated swimming pools. Other factors that must be taken into consideration for all cases include: time elapsed between death/discovery of the individual and collection of samples; and hospitalization following discovery with death occurring later during hospitalization.

**Drowning, Sphenoid Sinus Fluid, Chloride Level**

## G33    Recovery of Transplantable Organs After Cardiac Arrest in France

*Nathalie Jousset, MD\*, Arnaud Gaudin, MD, Damien Mauillon, MD, and Clotilde G. Rougé-Maillart, MD, CHU, Service de Médecine Légale, 4 rue Larrey, Angers, 49000, FRANCE*

After attending this presentation, attendees see an explanation of French legislation and summarize ethical problems linked to transplantation of organs coming from non-heart beating donors (NHBD).

This presentation will impact the forensic community by comparing situations, legislation, ethical problems, between countries concerning NHBD transplantation.

After being abandoned around the end of the 1960s, the transplantation of an allograft recovered after cardiac arrest has been resumed again in France in 2006 (decree of 2 August 2005: art R.1232-4-1,2 and 3 of the public health code).

Recently, according to the international scale, five situations that could lead to the recovery of transplantable organs after cardiac arrest were identified according to a classification called "Maastricht" which describes the potential donors. In France, the donors of class III (cessation of all medical care) were excluded.

To achieve an effective transplantation, the donor has to be legally dead in the eyes of the law, and the organs still viable medically. A legal definition of death, in the purpose of the recovery of the transplantable organs of the "dead" donors comes up against this contradiction.

In front of this issue, certain countries recommend against giving a legal definition of the death criteria. It is not the orientation chosen by France that continues to attempt to define a legal framework in order to obtain the society acceptance of the recovery of transplantable organs of "dead" donors.

Despite this, ethical questions arrise. Are criteria adopted to define death enough? What is the place of non-heart beating donor transplantation with new technical resuscitation as extracorporeal life support for prolonged cardiac arrest? How does family and medical staff support this protocol?

**Non-Heart Beating Donors, Legislation, Ethical Reflection**

## G34    Veterinary Forensic Science: Documentation, Processing, and Interpretation of Physical Evidence at Scenes of Animal Crimes

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After attending this presentation, attendees will gain an understanding of the current issues faced by prosecutors, judges, law enforcement officials, and veterinarians when attempting to bring cases of animal cruelty and death to trial in the courtroom. Participants will also gain a more detailed understanding of how modern forensic science as practiced at scenes of human death can be readily applied to the animal crime scene.

This presentation will impact the forensic community by giving a more detailed description of current problems and issues involved in the application of forensic science techniques to scenes of animal crime. Additionally, the participant will gain an improved understanding of the needs within the veterinary community and will be better prepared to utilize their own knowledge and forensic specialty to provide assistance to those in the veterinary forensic science community.

With the passage of many animal cruelty laws, the need to apply current forensic science methodologies to these investigations has increased dramatically. Animals have many similarities to humans in their response to traumatic injury with some noted exceptions. Forensic science techniques utilized at human crime scenes and applied human victims can often be easily applied to scenes involving cruelty or death to animals. This symposium will address the application of forensic techniques developed for the investigation of human death to animal cases and unique findings at animal crime scenes.

**Veterinary Science, Veterinary Forensics, Animal Cruelty**

## G35 Natural Causes of Sudden Unexpected Infant Death: A Seven Year Retrospective Forensic Autopsy Study in Hubei, China

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After attending this presentation, attendees will become familiar with the common natural causes of sudden unexpected infant death in Hubei province, China and will better understand the difference in the diagnosis of sudden infant death between China and western countries.

This presentation will have an impact on the forensic science community as it suggests that further studies are needed to focus on the differences in the diagnosis of sudden infant death between developing countries and developed countries.

The importance of a forensic investigation and autopsy in cases of sudden infant death has only recently received attention in China. An analysis of forensic autopsy data on sudden infant deaths in Hubei, China has never been undertaken. This report describes the epidemiological characteristics and pathological findings of sudden infant death cases investigated by the Department of Forensic Medicine at the Tongji Medical College in Hubei, China from 1999 to 2006.

A retrospective study of forensic autopsy cases conducted at the Department of Forensic Medicine, Tongji Medical College in China over a seven year period between1999 and 2005 yielded a total of 68 infants who died suddenly and unexpectedly in Hubei province. The age ranged between newborn and 12 months. A total of 41 cases (60%) of the deaths occurred in the neonatal period, 13 (22%) infants in the first six months of life, and the remaining 12 cases (18%) in the age between seven months and one year. There were 54 males and 14 females (M: F = 3.8:1). The most common cause of sudden neonatal death was pneumonia (N=14), followed by congenital abnormalities (N=9); asphyxia due to amniotic fluid aspiration (N=7); respiratory distress of newborn (N=3); intrauterine hypoxia and birth asphyxia (N=3); complications of prematurity (N=2); newborn affected by complications of cord (N=1), birth trauma (N=1); and tetanus (n=1); and one death with undetermined cause.

The three leading causes of sudden death in infants, age 1 to 12 months were pneumonia (N=11), congenital heart disease (N=3), and meningitis (N=2). Only one infant was diagnosed as SIDS death.

Infectious diseases are a frequent cause of death in infants who died suddenly and unexpectedly in Hubei, China. These findings contrast with those from developed countries in which Sudden Infant Death Syndrome is the commonest cause of sudden unexpected death in infancy. This study demonstrates that it is important to document autopsy-based data such as these in the planning of medical services in a developing country.

**Sudden Infant Death, Forensic Investigation, Autopsy**

## G36 Biomedical Engineering in Root Cause Analysis – Example: Assessing Infant Apnea-Related Deaths

*Bruce H. Barkalow, PhD\*, William E. Grant, MA, and Farrah J. Curran, BS, B.H. Barkalow, PC, 490 Quarterline St., Newaygo, MI 49337-9125*

After attending this presentation, attendees will gain a better understanding by example of how Biomedical Engineering can assist in root cause analysis by examining how testing of subject apnea monitors and analyses of downloaded patient data can be useful in determining device failure versus human error.

This presentation will impact the forensic community by demonstrating how Biomedical Engineering analysis can shed light on important information involving medical devices. In this example, testing of subject apnea monitors and analyses of downloaded patient data can be useful in determining whether such devices have failed, or whether other factors (including human error) led to infant deaths.

Infants (primarily those of low birth weight) who are at risk for Sudden Infant Death Syndrome (SIDS) are often prescribed apnea monitors for at-home use. Infant apnea monitors are designed to alert caregivers if a child has become apneic and/or has heart rate changes outside of the preset limits. These monitors are not fool proof, however, and every year some children who are being monitored die. When this happens, it is the responsibility of the Medical Examiner to ascertain why the death occurred. Biomedical Engineers trained in this technology can play a vital role in these death investigations.

Apnea in neonates and infants occurs most likely because of immaturity of their respiratory and neurologic systems. Though it is common for infants to pause in their breathing for short periods, pauses lasting longer than 20 seconds are cause for concern, as are pauses of shorter duration accompanied by decreased heart rate. Infant apnea monitors are designed to detect increases and decreases in heart rate along with pauses in breathing, and sound an alarm if they occur. This is accomplished by attaching a belt with a series of electrodes around the infant's chest. The electrodes are attached to the monitoring unit itself. Monitors should have a battery backup, a remote alarm, a power loss alarm, a battery charge or AC power indicator, a sibling alarm as well as an internal memory for event and physiological data storage.

In cases where a child monitored with one of these devices has unexpectedly expired, a technical analysis should be performed by a trained Biomedical Engineer. A physical examination of the monitor itself should be conducted, along with an assessment of the electrical circuitry and analysis of monitor-downloaded data. A combination of downloaded patient and monitoring compliance data from the apnea monitor memory can be cross-correlated with events, such as feeding schedules or EMS run sheets. Deaths of monitored infants have been related to such issues as monitor hardware and software failures, obstructive apnea (often not detected), parental monitoring compliance, inability to hear the alarms, cardiac artifact in the transthoracic impedance signal, or electromagnetic interference, to name a few. If an apnea monitor is sent to the Medical Examiner's office along with an infant who has expired, it should be maintained as evidence, and the stored data downloaded and analyzed along with statements of the caregivers.

Case material corresponding to several of these failure-related issues will be presented to illustrate how a root cause approach can assist in making sense of why such tragic events may have occurred.

**Biomedical Engineering, Infant Apnea Monitor, Sudden Infant Death Syndrome (SIDS)**

## G37  *In Utero* Traumatic Head Injuries During a Motor Vehicle Collision: Case-Report and Review of the Literature

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After attending this presentation, attendees will understand the importance of fetal brain injury in utero after a motor vehicle collision which can lead to hypoxia, direct impact and acceleration-deceleration injuries. A discussion of maternal restraint will also be presented.

This presentation will impact the forensic community by highlighting the possible neurologic complications associated with motor vehicle collision and the fetal brain in utero, especially in relation to acceleration-deceleration forces

Fetal brains differ from neonatal and infantile brains in development and the environment surrounding them, namely the protection offered by the amniotic fluid, uterus, and maternal abdominal wall. Head injuries inflicted during motor vehicle collision result from both direct impact and from acceleration-deceleration forces. The effects of these forces on the fetal brain and eyes are poorly described in the literature. The American College of Obstetrician and Gynecologists and the National Highway Traffic Safety Administration recommend that pregnant women use a 3-point restraint system with the lap belt positioned under the uterus based on the hypothesis that the amount of fetal head acceleration and abdominal force is significantly reduced.

A 32-week pregnant, 31-year-old Hispanic woman was the restrained front seat passenger in a mini van that was rear-ended by a full sized tractor-trailer. Following the collision she was alive but in a deep coma, tachycardiac and with a blood pressure of 119/62. She was intubated in the field and transported to the hospital. Fetal heart monitoring revealed 40 beats per minute. An emergency C-section was performed slightly more than one hour following the impact, and a 1,580 gram baby girl with Apgar scores of 0, 0 and 0 was retrieved. Examination of the uterus revealed placental abruption. Maternal injuries detected by CT included a complete torso passenger-site shoulder and lap seat-belt contusion with the lap section located on the pelvis, C4-C5 fractures with bilateral internal carotid injuries, fractured ribs, pneumo and hemothoraces, liver lacerations, fractured T3 and L1 through L5 vertebrae, retroperitoneal hematoma and acetabular fractures. The mother was pronounced dead two and a half hours after the collision. A complete postmortem examination of the stillborn female was performed including neuropathologic and ophthalmic pathologic assessment. The findings obtained at the autopsy of the stillborn, which will be presented, included the external and internal traumatic injuries, head and spinal injuries, including direct impact and acceleration-deceleration injuries to the brain, spinal cord, and eyes, grossly and microscopically. These findings will be discussed in relation to previous literature reports and the seat-belt recommendations for pregnant women.

Hypoxia, direct impact, and acceleration-deceleration forces are the usual components producing complex neuropathologic injuries. The resulting lesions depend on the age of the victim; the susceptibility of the immature brain to trauma and the resulting pattern of injuries differ between fetal, childhood and adult brains. There are few reports in the literature of traumatic fetal brain injuries resulting from motor vehicle collision, rare reports including autopsy or eye pathology findings. In addition, findings are correlated with the use of a 3-point restraint system with the lap belt positioned under the uterus as recommended.

**Fetus, Brain, Traumatic**

## G38  Return of an Old Acquaintance - A Case of Septic Abortion

*Karen B. Looman, DO\*, Hamilton County Coroner's Office, 3159 Eden Avenue, Cincinnati, OH 45219*

After attending this presentation, attendees will become acquainted, or reacquainted, with septic abortion and some of the associated anatomical and microscopic findings.

This presentation will impact the forensic community by reminding everyone of a time when illegal abortions, septicemia and death were more common in the United States. After Roe vs. Wade, the frequency of cases dwindled. New and seasoned medical examiners may benefit from a reminder of what to expect in a septic abortion case, especially in an ever changing political arena.

On January 22, 1973, the Supreme Court decision regarding Roe vs. Wade allowed women to have legal medical abortions in the United States. In the past, women who were seeking an abortion for a reason other than to protect the health of the mother were limited to often questionably sterile and clandestine procedures. Cases of septic deaths from improperly performed procedures and post-operational infections were high. The numbers of such cases dropped off dramatically after the landmark legal decision. Some of the more experienced medical examiners may remember physical and microscopic findings in such cases. Less experienced doctors should be aware of some of the possible findings in a septic abortion case.

A 31-year-old married woman, with four living children, was rushed to a local hospital after becoming hypotensive at a small medical clinic. She was presented to the clinic with a complaint of food poisoning which included nausea, vomiting, diarrhea, and malaise. The clinic diagnosed pyelonephritis and gave her intramuscular ceftriaxone. Within minutes, the woman complained of tongue swelling, shortness of breath, and hypotension likely indicating an allergic reaction.

The question of an allergic reaction to the antibiotics was never specifically addressed during her emergent stay in the hospital. The emergency room doctors noted erythema and tense swelling of the woman's lower extremities. A serum pregnancy test was positive with a human chorionic gonadotropin (hCG) level of 6124 mIU/ml (2-3 week gestation). She was transported to the intensive care unit in extremis. She was intubated and went into cardiac arrest. She was resuscitated once, but a second cardiac arrest was fatal.

Significant medical history went back approximately one month prior when the woman was involved in a motor vehicle collision and broke her foot. On her two week follow up, her primary doctor asked her if she had had a pregnancy test in the ER at the time of the accident. The woman said yes and that she had been on her menses. A thorough record check did not show a pregnancy test was performed. On the day of the terminal event, she had a tampon in place, noted on the pelvic exam.

The woman's mother reported that her daughter was going through a "mid-life" crisis and acting wildly. She got several new tattoos and was having an affair. The mother stated it was entirely possible she became pregnant while having the affair because her husband had a vasectomy.

At autopsy, the tense swelling and erythema of the lower extremities was noted to be in a trouser distribution. The legs had a crepitus-like feel to them. Internal examination of the pelvis found that the uterus had a slightly purple and erythematous fundus but no perforation. The endometrial cavity was full of hemorrhagic debris but no obvious fetal tissue was seen grossly. Microscopic examination of the debris revealed extensive autolysis with a few fragments resembling placental villi. Blood cultures had been performed at the hospital prior to the cardiac arrest. Despite having received one dose of antibiotics at the medical clinic, one of the blood cultures grew *Clostridium perfringens*, which can produce gas gangrene.

Putting the story together, it appeared the woman got pregnant and either had an abortion or a spontaneous miscarriage with retention of products of conception. There was no history she had been to any doctor

for an abortion. Neither her mother, friends, nor family knew anything about her being pregnant. If the decedent did get an abortion through a clinic or through personal instrumentation, there were complications that were not addressed. If the uterine contents were retained products of conception from a miscarriage, the sepsis would be explained. There is no confirmation of her pregnancy before the last ER visit because a pregnancy test was not performed at the time of her car accident. The sepsis caused a trouser distribution of swelling and erythema of her lower extremities. The crepitus-like feel of the legs may be indicative of gas gangrene from Clostridium. Cause of death in this case is acute sepsis due to uterine infection.

It is important to note that it would be unusual to find identifiable fetal or placental tissue in a case such as this. The time that is required for the development of the sepsis is long enough for all such tissues to autolyze and be unidentifiable under the microscope. Interviews with family and friends regarding medical and social history are very important in understanding the background of the illness. Also helpful, are discussions with other medical examiners with more experience that have seen a few of these cases. Their wisdom is invaluable.

**Abortion, Miscarriage, Septicemia**

## G39 Fatal Rupture of Splenic Artery Aneurysm in a Pregnant Woman With Portal Hypertension

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By attending this presentation, attendees will learn about important pathological characteristics of splenic artery aneurysms, their causative correlation with pregnancy and portal hypertension, as well as clinical and medicolegal aspects of cases where their rupture leads to a fatal outcome.

This presentation will impact the forensic community by demonstrating how development of splenic artery aneurysm may be connected with pregnancy and portal hypertension; it is a potential source of profuse abdominal hemorrhage and sudden unexpected death, sometimes in previously apparently healthy individuals. Also of importance in this particular case is the patient's intention to hide a known disease from the attending physician, which may cause serious and potentially fatal errors in medical treatment.

Fatal complications of pregnancy and childbirth always attract special public and medical attention and are usually a serious challenge for forensic pathologists, especially if death occurs suddenly and unexpectedly in a previously apparently healthy woman. This case concerns a 30-year-old female, five months pregnant with her first child, who was found dead in her flat. According to the statement of the husband, cited in the initial police report, she had regular check-ups with her obstetrician. During pregnancy she did not complain of abdominal pain or any other discomfort. On the day in question the husband left the flat at 7:00 p.m. while she stayed at home preparing a meal. When he came back one hour later he found her lifeless, lying on the bed in their bedroom. He immediately called an ambulance; they arrived promptly and attempted CPR but to no avail; she was declared dead at the scene. The cause and manner of death were undetermined and the examining magistrate requested a medicolegal autopsy.

The postmortem examination showed a female of moderate physique, 167cm in height, with external signs of pregnancy in keeping with the gestational age of five months. The skin and conjunctivae were very pale and hypostasis was poorly developed. There was evidence of attempted resuscitation. There was no evidence of external trauma. Internal examination of the cranial and chest cavities revealed only pallor of all organs and tissues, but no other significant pathological findings. Opening of the abdomen showed about 4500 ml of blood in the peritoneal cavity. Examination of the uterus and adnexa showed no evidence of ruptures or other possible source of bleeding. Within the uterine cavity was a dead female fetus, normally developed according to gestational age. The other possible site of hemorrhage was an extremely enlarged spleen, which weighed 780 g, but showed no evidence of rupture. Finally, the source of bleeding was discovered by examination of the splenic artery, which was dilated throughout its course (with a circumference measuring up to 1.5 cm), tortuous, with one 1 cm long fusiform and two big saccular aneurysms, measuring 4 cm and 2 cm in diameter. The larger of the two saccular aneurysms showed a 0.5 cm long rupture, while attached to the smaller of the two was an accessory spleen, measuring 1 cm in diameter. There were several further accessory spleens in the vicinity, with a diameter varying between 0.5 cm and 1.5 cm. The portal vein was almost completely obliterated by an old partly calcified thrombus. Death was deemed natural, caused by exsanguination due to a ruptured splenic artery aneurysm. During interview with the deceased's husband it became apparent that all the above mentioned severe pathological changes involving the splenic artery, spleen and portal vein, with portal hypertension, had been diagnosed both radiologically and clinically two years prior to the fatal outcome. According to the deceased's medical records, this important anamnestic information had not been disclosed to the obstetrician who controlled her pregnancy.

The important pathological, clinical, and medicolegal issues concerning the reported case, mainly the causative relationship between pregnancy, portal hypertension, and splenic artery aneurysm, clinical recommendations regarding pregnancy in women with diagnosed splenic artery aneurysm as well as medicolegal problems connected to patient-physician relationship, and potential accusations of medical negligence and malpractice will be discussed.

**Splenic Artery Aneurysm, Pregnancy, Portal Hypertension**

## G40 How Often is Pre-Existing Disease Found in Child Deaths?

*M.G.F. Gilliland, MD\*, Brody School of Medicine at East Carolina University, Pathology & Laboratory Medicine, Brody Building, Greenville, NC 27858-4354*

After attending this presentation, attendees will be able to describe the frequency of finding pre-existing disease in a group of child death investigations and discuss the potential significance of such findings in individual cases.

This presentation will impact the forensic community by demonstrating why the forensic science community needs a scientific basis for comparison of individual cases to a larger group of child deaths when providing information to triers of fact.

Forensic pathologists are often asked whether injured children have pre-existing diseases when discussing findings in death investigations. Commonly the pathologist is then asked to compare individual case

findings with findings in a larger population of children. The objective of this study was to review a series of child death investigations and determine the frequency of identifying pre-existing disease in the group.

A prospective study focused on the deaths of 169 of approximately 400 child deaths investigated by the Southwestern Institute of Forensic Sciences (SWIFS) in Dallas, TX from 1981-1989. Investigation of these deaths included information about the circumstances of death or collapse, prior medical and social history, autopsy examination with ocular examination, toxicologic investigation, radiography when indicated, and additional investigations when questions remained. The study has been previously described and included: 19 asphyxial, 80 closed head injury, 13 trunk injury, 13 central nervous system disease, 13 sudden infant death syndrome, 21 other natural deaths, and 10 deaths with undetermined cause and manner. The central nervous system diseases included meningitis, seizure disorders, spontaneous intraventricular and subarachnoid hemorrhages, and a brain tumor. The other natural deaths included respiratory tract illnesses, sepsis, congenital heart disease, myocarditis, a volvulus, and a dehydration death. Demographics were similar to the child deaths investigated at SWIFS: 78% were two years of age and under; 98 were white, 51 black, 16 Latino, and 4 other ancestry. Over half, 59%, were boys. Pre-existing disease was defined as diseases found at autopsy whether the disease contributed to death or not. However, children with diseases resulting from cardiovascular collapse were not included in the pre-existing disease group. Bronchopneumonia, myocardial ischemia, or watershed infarcts were found in some of the children who were well until an injury event occurred. These diseases were considered consequences of the collapse event and not included. Analysis of the data regarding bronchopneumonia has previously been reported for this study population. Review of the 169 deaths identified 60 children with pre-existing diseases and 109 without such diseases.

The group was further subdivided by the mechanism of the immediate cause of death. The distribution of pre-existing disease among unnatural, natural, and undetermined causes revealed:

| Manner of Death | Pre-Existing Disease Present | Absent | Total | % of Subgroup with Pre-Existing Disease |
|---|---|---|---|---|
| Inadvertent | 6 | 30 | 36 | 17% |
| Intentional | 16 | 60 | 76 | 21% |
| Natural | 34 | 13 | 47 | 72% |
| Undetermined | 4 | 6 | 10 | 40% |

Pre-existing disease was uncommon among injured children. For some of the intentional injury deaths investigation suggested that otitis media or retardation may have been a factor in increasing the caregiver's frustration with the child. The pre-existing diseases did not appear to increase the risk of injury in the inadvertent injury deaths and appeared to be incidental findings. The deaths attributed to natural causes identified diseases sufficient to account for the children's deaths, and, as such, had the greatest frequency of pre-existing disease by the definition used in this study. The 13 with no pre-existing disease included nine of the SIDS deaths which did not have sufficient disease or injury to account for deaths. Review of scene and circumstances, medical records, and search for social service involvement revealed no concerns. In the 1980s such non-suspicious deaths were attributed to Sudden Infant Death Syndrome at SWIFS. The other four natural deaths with no pre-existing disease were diseases resulting from a prior remote injury from which the child had at least partially recovered. Undetermined deaths in this study had neither adequate natural disease nor injury to account for the deaths and suspicious scenes, circumstances, medical records, or social service histories.

Review of a series of child deaths including both natural and unnatural causes and manners of deaths revealed that most natural deaths occurred in children with pre-existing disease and most unnatural deaths occurred in otherwise healthy children. Reporting such findings provides a scientific basis for comparison of individual cases to a larger group of child deaths.

**Child Death, Child Abuse, Pre-Existing Disease**

## G41 Lymphogenic Cardiomyopathy: A Possible Cause of Non-Immune Fetal Hydrops

*Antonio Bonifacio, MD\*, Stefania Zerbo, MD, Cettina Sortino, Pierangela Fleres, MD, and Emiliano Maresi, PhD, Department of Biotechnology and Legal Medicine, Section of Legal Medicine, Via del Vespro, n. 129, Palermo, 90127, ITALY*

After attending this presentation, attendees will gain knowledge of three particular cases of congenital and isolated cardiac lymphangiectasia manifested *in utero* with cardiac failure and hydrops.

This presentation will impact the forensic community by exploring how cardiac isolated lymphangiectasia might represent a new nosological entity that should be included among the primary cardiomyopathies (lymphogenic cardiomyopathy). Consequently, this entity should be investigated among the possible causes of non-immune hydrops foetalis (HF).

It is the intent of this presentation that cardiac isolated lymphangiectasia might represent a new nosological entity that should be included among the primary cardiomyopathies (lymphogenic cardiomyopathy). Consequently, this entity should be investigated among the possible causes of non-immune hydrops foetalis.

HF is an aspecific and terminal sign of many fetal diseases that could be observed at any time during pregnancy. In fully developed HF, there is subcutaneous oedema with fluid accumulations in peritoneal, pleural, and pericardial cavities. The umbilical cord and placenta are also oedematous and there is polyhydramnios. In the early stages of HF, the fluid accumulations are not present in all compartments. HF is caused by three main mechanisms: anemia, hypoproteinemia, and cardiac failure. Most cases fit within this classification, although some cases remain unsolved under the name of "idiopathic HF." Another classification divides HF into treatable (27%) and untreatable (73%) forms. The success of isoimmunization prevention programs demonstrated that most cases of HF are now non-immune and depend on cardiovascular diseases (22%), chromosomal abnormalities (13%), thoracic causes (10%), anemia (homozygous α-thalassemia), monochorionic twinning (6%), infections (5%), miscellaneous (16%), not determined (20%). Cardiovascular HF seems to be more frequently associated with structural and functional abnormalities that cause volume and/or pressure overload on the right atrium such as left heart syndrome, arrhythmias, myocarditis, cardiomyopathies, cardiac tumors, myocardial infarction, and arterial calcification.

Three unusual cases of congenital and isolated cardiac lymphangiectasia (ICL) manifested *in utero* with cardiac failure and hydrops will be presented.

**Case1:** A male hydropic fetus with a gestational age of 14.2 weeks without dysmorphia. The mother was 32-years-old and had four pregnancies, one of them resulting in miscarriage due to unknown causes. Ultrasound of the fetus and placenta showed regular heart rate with biventricular hypocontractility and without congenital cardiac and extra-cardiac defects and polyhydramnios. Amniocentesis revealed a normal karyotype.

**Case 2:** A male hydropic fetus with a gestational age of 19.5 weeks without dysmorphia. The mother was 29-years-old and had a previous miscarriage due to a premature rupture of the placental membranes (acute chorioamnionitis) at the 25th week of gestation. Ultrasound of the fetus and placenta showed regular heart rate with biventricular hypocontractility, without congenital cardiac, and extra-cardiac defects, and polyhydramnios. Amniocentesis revealed a normal karyotype.

**Case 3:** A female non-hydropic fetus with a gestational age of 22 weeks without dysmorphia, except for the presence of a single head held plica. The mother, 27-years-old, was at first pregnancy. Ultrasound of the fetus and placenta showed light pericardial effusions, regular heart rate with biventricular hypocontractility, without congenital cardiac and extra-cardiac defects, and polyhydramnios. Amniocentesis revealed Trisomia 21.

In all cases, fetal autopsies showed ultrasound findings conducted during pregnancy. At histology, the organs were normally structured except for the heart that showed a "moth-eaten" aspect in the ventricular walls, due to severe, diffuse and transmural lymphangiectasia and interstitial lymphedema. The interposed myocardium resulted compressed, distorted, trabeculated, and with multifocal patchy coagulative miofibrillolisis (contraction band necrosis). The morphological examination in situ of apoptosis highlighted in all cases the presence of frequent apoptotic events in the endothelia of small arteries and veins.

**Discussion:** ICL is an extremely rare entity and up-to-date the literature reports describe only one case characterized by septal localization of this lesion causing septal hypertrophy and left ventricular outflow obstruction mimicking hypertrophic cardiomyopathy. These cases represent the first report of ICL involving diffusely the heart, causing cardiac failure and hydrops of various degrees. The findings of a marked apoptosis in the endothelial cells of blood vessels suggest that interstitial lymphoedema and lymphatic overload is due to increase vascular permeability of the cardiac blood microcirculation.

**Hydrops Fetalis, Lymphangiectasia, Cardiomyopathy**

## G42 Prolonged Survival Time Following Duodenal Transection in a Child With Abdominal Trauma

*Lauren E. Moser, MD\*, Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201; Mary G. Ripple, MD, 68 Bluebird Road, Port Deposit, MD 21904; and David R. Fowler, MD, Office of the Chief Medical Examiner, 111 Penn Street, Baltimore, MD 21201*

After attending this presentation, attendees will be able to recognize the symptomatology associated with abdominal trauma and will be able to understand the correlation between symptoms and pathologic injury correlating histologic examination of tissues with a timeline of events.

This presentation will impact the forensic community by providing valuable information about symptomatology associated with blunt abdominal trauma. Seemingly minor external injuries may harbor terminal internal pathology. Recognition of the potential severity of these injuries may prompt clinicians to conduct more thorough patient examinations and pursue imaging studies to identify unexpected internal injuries. In many cases of homicidal blunt force injury, forensic pathologists are asked to estimate the time of injury despite the uncertain circumstantial timeline of case investigation. In this case, a relatively accurate timeline was known. Hence, the correlation with microscopic sections of the injury can provide assistance in the evaluation of previously published timelines for the inflammatory response.

Upon completion of this presentation, attendees will have an appreciation for the clinical symptomatology associated with duodenal transection following blunt abdominal trauma and the importance of histologic evaluation of this type of injury to foster the sequencing of events. Subtle symptoms can dissimilate potentially emergent, life-threatening pathology. This particular case discussion exemplifies prolonged (approximately 24 hrs) survival in a child that sustained a duodenal transection injury after falling from a bicycle. A literature review of comparable cases will also be performed. Case correlation may aid in establishing a time range of survival, which would portend significant clinical value. Clinicians who are knowledgeable about the potential injuries caused by abdominal trauma are more likely to suspect injury despite the absence of suggestive symptoms. Such analysis will likely demonstrate that an official clinical diagnosis of intestinal laceration occurs when an individual's symptoms are more severe (postulating that there is a period of survival status-post injury).

A 9-year-old Hispanic female sustained head and abdominal injuries after falling from her bicycle on 6/27/08 at approximately 1-2 p.m. She was examined and released from a local hospital without having undergone imaging studies. According to the report, later that evening, the child began vomiting and subsequently went to sleep. The next morning, she continued to feel nauseous and vomited in the morning. She went to bed around noon and was found unresponsive at 12:50 pm on 6/28/08. The child was pronounced dead at 1:47 pm on the same day. Investigation revealed no evidence of anything other than unintentional injury. Autopsy revealed a laceration of the head with an underlying depressed skull fracture and focal epidural hemorrhage; focal minor contusions and abrasions of the torso; hemoperitoneum; duodenal transection distal to the pylorus; contusion of the liver; intra-abdominal soft tissue hemorrhage of the ligamentum teres, greater and lesser omentum, and mesentery; petechial hemorrhages of the lower lobe of the right lung; peri-pancreatic soft tissue hemorrhage with bile staining; and minor abrasions and contusions of the extremities. Histologic examination revealed an abundance of neutrophils, fibrinous debris, scattered monocytes, and an absence of hemosiderin laden macrophages, which confirmed the timeline of investigation. The cause of death in this 9-year-old female was head and abdominal injuries sustained after falling from a bicycle.

A common cause of accidental abdominal trauma in grade-school children is due to impact with bicycle handlebars. This injury can mimic homicidal blunt force injury. The importance of histologic examination of injuries in different tissues with a known timeline of events aids in predicting an unknown timeline in homicidal blunt force injury cases. Such trauma commonly causes lacerations of the duodenum, and in many instances, severe internal organ damage is accompanied by a dramatic paucity of significant external injury. Abdominal organ injury has a poor prognosis due to delay in therapy. An appreciation for the potential severity of blunt abdominal trauma, which can provoke more efficient diagnosis of the injury and hastened therapy, may save a precious life.

**Duodenum, Trauma, Survival**

## G43 Role of Scene Reconstruction in the Medicolegal Investigation of Sudden Unexpected Infant Deaths

*Richard C. Harruff, PhD, and Pamela S. Ulmer, DO\*, King County Medical Examiner's Office, 325 9th Avenue, HMC Box 359792, Seattle, WA 98104-2499*

After attending this presentation, attendees will recognize the value of scene reconstruction as a routine component of infant death scene investigation.

This presentation will impact the forensic community by showing how medicolegal death investigators can enhance the quality of their scene investigations and provide valuable information that may be used to prevent or reduce future infant deaths.

Complete investigation of sudden unexpected infant deaths requires scene investigation, full autopsy, and review of the case history. Careful scene investigation is crucial, not only for understanding why one particular infant died, but also for developing valid strategies to prevent future infant deaths. This presentation examines the techniques and value of doing scene reconstruction as part of a rigorous investigation of a sudden unexpected infant death.

The King County Medical Examiner's Office (KCMEO) investigates all sudden unexpected infant deaths using: (1) a standardized scene investigation protocol, (2) a complete autopsy including microscopic examinations, toxicology, metabolic screening, and microbiological cultures when indicated, and (3) review of the case history with police and child protective agencies. For this study, the computerized KCMEO database from 1995 to 2008 was searched for all deaths of children between the ages of one week and three years. These

were then analyzed to group the deaths by manner of death and further subclassify natural death. In addition, the photographic records of KCMEO were individually reviewed to find cases in which scene investigation included scene reconstruction. The cases in which scene reconstruction yielded information important for certifying cause and manner of death were then selected as examples to demonstrate the techniques of this investigative tool and its value for the overall death investigation.

Between 1995 and 2008, 505 deaths of infants and young children from one week to 3 years of age were recorded in the KCMEO database. Of these 505 deaths, 326 of these were classified natural, 107 accident, 2 complication of therapy, 47 homicide, and 23 undetermined. There were 232 deaths that were certified as SIDS. During this time period, 151 deaths were investigated using a doll or similar prop to reconstruct the scene in which the caregiver found the child dead. The scene reconstruction included instances of natural deaths, accidents, and deaths certified as undetermined.

Scene reconstruction is an essential part of the investigation into the death of an infant or young child. Invariably the death scene is disturbed and therefore requires a patient, well-trained, experienced, and compassionate investigator to uncover the details surrounding the death. Using a fabricated, stuffed doll or similar prop, and working patiently with the caregiver is the best means for establishing the location and position in which the child was last seen, the usual position for sleeping, and the position when found unresponsive. Furthermore, this method supports a photographic record that is fairly acceptable to the caregiver(s) and that can later be used to demonstrate the death scene in an emotionally neutral manner. Risk factors and hazards, including bed sharing, present in the child's environment can be readily documented and demonstrated with respect to the specific hazard and the way in which the hazard is responsible for the death. Witness reliability can also be assessed with this technique. As important as scene reconstruction proves to be, there are several obstacles in utilizing this method. Emergency medical personnel frequently disrupt the integrity of the scene by transporting babies that they know are dead to the hospital emergency department; this practice must be strongly discouraged. The death investigator, agency, or otherwise well-meaning individuals may feel that scene reconstruction is too invasive into the caregiver(s) grief and privacy. Training, experience, and compassion are needed to overcome this obstacle. There is the valid concern that a caregiver or witness may not be reliable in reconstructing the scene for the investigator(s). Again, experience has shown that patience and compassion are the most valuable qualities for gaining witness trust and revealing the truth. In conclusion, scene reconstruction provides invaluable information, and therefore, should be considered one of the standards for a quality infant death investigation.

**Medicolegal Investigation, Sudden Infant Death Syndrome, Scene Reconstruction**

## G44 Death by INR: A Case of Vitamin K Deficiency Bleeding Masquerading as Shaken Baby Syndrome

*O.C. Smith, MD\*, Conscience and Science in Medicine, LLC 9639 Rosemark Road, Atoka, TN 38004; and Jennifer Griffith, MS, Lani Collins, MS, and Linda Williford, PhD, The University of Tennessee Clinical Laboratory Sciences, 930 Madison Avenue, Memphis, TN 38163*

After attending this presentation, attendees will understand the need for a deconstruction oriented approach when evaluating Shaken Baby Syndrome cases; realize that differential diagnoses exist for most any medical finding and that superficial observations and failure to develop the differential can lead to diagnostic errors and wrongful process.

This presentation will impact the forensic community by giving a better understanding of the value of integrated investigations and their ability to serve justice.

A 7-month-old child died at a pediatric hospital of "non-accidental trauma." The chart was given to the pathologist, and conversation from the treating physician indicated intracranial and retinal hemorrhaging. The pathologist recorded a cryptic entry: "INR=1.1." An extensive medical chart dating back to the decedent's third day of life was not disclosed.

The decedent was the twin B of twins born at 35 1/2 weeks, discordant from his twin sister by a birth weight 20% less. Severe reflux disease was present since birth. He required a fundoplication and a feeding tube through the abdomen. At birth he was in the 25th percentile, until his sixth month when it was in the 15th and terminally, had fallen to the 5th. Despite adequate nutritional intake, he no longer absorbed the nutrients and was diagnosed with failure to thrive. Shortly before death, he had the gastrostomy site cauterized for continual bleeding.

The medical examiner ruled Shaken Baby Syndrome. The father was arrested subsequent to his statement that after seeing his son on the floor with his aggressive 13-month-old daughter kneeling on the infant's stomach and her hands at his neck; he separated them and found the infant struggling to breathe. To revive the child, he "shook" it. He was charged with first degree murder. The defense desired a medical review.

Deconstruction revealed two different autopsy protocols, the absence of an adequate neck dissection, the missed presence of prior retinal hemorrhages, and the failure to observe a tongue tumor. The presence of the gastrostomy and fundoplicaton went unexplained, as did contusions the hospital reported, on the back. Significant hospital laboratory values included coagulation studies with a prolonged prothrombin time (PT) and a normal activated partial thromboplastin time (aPTT). The International Normalized Ratio (INR) was 1.1, within normal range. The timeline revealed an initial retinal examination with hemorrhage in the left eye, hours later both retinas were hemorrhagic. Iron stains of the eyes by the defense were positive, indicating remote hemorrhage. Records of the organ procurement organization indicated the use of vasopressors and anticoagulants, increasing the hemorrhages.

The differential diagnosis when the PT is long and the aPTT is normal is divided between liver disease or a deficiency of vitamin K an essential vitamin that enables the liver to produce coagulation factors. Vitamin K Deficiency Bleeding (VKDB) is a third world disease. The clinician relied upon the INR that no coagulopathy was present because the INR was normal. This is an inappropriate practice as the INR is intended *only* for those patients on coumadin therapy for periods greater than two weeks.

Studies from the University of Tennessee confirm clinicians rely upon the INR as an indicator of normal coagulation status. This practice obscures the initial stages of coagulopathies. Ironically, other studies ordered were not followed up, or were cancelled after death, thus preventing a definitive answer. The pediatricians remained adamant the child was murdered. The defense presented this finding to the prosecution, and settled via diversion.

**Shaken Baby Syndrome, Deconstruction, Coagulopathy**

## G45 Malicious Use of Nonprescription Cough and Cold Medications in Children

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After attending this presentation, attendees will understand the process and outcomes of a systematic evaluation of pediatric deaths associated with nonprescription cough and cold drugs and will gain an awareness of the rare but apparent malicious use of these medications in children.

This presentation will impact the forensic community by increasing awareness of the malicious use of these drugs and encourage more detailed investigations into all pediatric deaths.

The safety of nonprescription cough and cold medication use in children less than 12 years of age has been questioned. To better understand the safety profile of these drugs, an independent expert panel reviewed available death reports of children less than 12 years of age with mention of a cough/cold ingredient that were obtained from three sources: English language medical literature (1949-2007), National Poison Data System (1983-2007) and Manufacturer Safety Records (1980-2007). The panel assessed the causal relationship between each reported drug and death using predetermined definitions for "Definitely Related", "Likely Related", "Possibly Related", "Unlikely Related", "Definitely Not Related", and "Unable to Determine". These definitions were based upon clinical course, drug exposure history, body fluid/tissue analytical evidence, and alternative cause of death. The panel also attempted to categorize the dose ingested (therapeutic/supratherapeutic), intent of administration (therapeutic/non-therapeutic) and potential contributing factors for all deaths determined to be at least possibly related to a cough and cold drug.

Of 227 fatality reports reviewed, 92 (41%) deaths were judged at least possibly related to one or more nonprescription cough and cold drugs. The panel determined that 79 of these involved a supratherapeutic dose of the suspect drug. The dose could not be assessed in the remaining 13 cases. There were no cases for which the panel suspected a therapeutic dose was involved. Of the 92 related deaths, 68 involved an overdose of the suspect drug administered by a caregiver. Ten such cases involved the administration of the drug in a daycare setting. Twenty-four reports (18 - age <2 years, 5 - age 2 to <6 years, 1 - age 6 to <12 years) indicated that the drug was given to the child without an appropriate therapeutic indication (i.e., not for cough or cold symptoms). The panel determined that malicious intent was a contributing factor in 20 cases. These were cases in which the drugs were used to either sedate or intentionally harm the child. Other signs of child abuse including hematomas and healed fractures were evident in some cases.

The incidence of non-accidental child injuries, specifically death, resulting from the misuse of nonprescription cough and cold medications or other drugs is unknown, in part because there is no standard laboratory testing during pediatric fatality investigations. Postmortem drug concentration levels are rarely reported because appropriate specimens are not routinely collected during autopsy. These body fluid/tissue drug levels are often difficult to obtain due to limited specimen and analytical challenges. They are also difficult to interpret due to postmortem effects including redistribution as well as limited peer-reviewed reference ranges for the pediatric population. Interpretation of drug levels should be done with pharmacokinetic parameters and must not rely on published ranges which do not take into account the elapsed time since administration of the drug. Those performing investigations of pediatric deaths, especially in children less than 2 years of age, should consider overdose of cough and cold medications and other drugs, including instances of malicious use of these drugs by parents or caregivers.

**Pediatric Deaths, Nonprescription Medications, Drug Toxicity**

## G46 Wrongful Convictions and Pediatric Forensic Pathology: The Canadian Experience

*Jeffrey R. Manishen, BA*, Ross & McBride LLP, 1 King Street West, 10th Floor, PO Box 907, Hamilton,, L8N 3P6, CANADA*

After attending this presentation, attendees will understand of how inadequate training and supervision, difficulties in the communication of opinion evidence, and problematic areas in pediatric forensic pathology may result in wrongful convictions. Those in attendance will enhance their understanding of the ways to improve the interaction between forensic scientists and the criminal justice system.

This presentation will impact the forensic community by exploring the recommendations of the Inquiry into Pediatric Forensic Pathology conducted by appellate court Justice Stephen Goudge in Toronto, Ontario, Canada, which are likely to have a significant impact on the prevention of miscarriages of justice on an international level.

As a result of the identification of serious errors in the postmortem reports and testimony of Dr. Charles Smith, a pathologist called by the prosecution in several child homicide cases in Ontario, Canada, the provincial government convened a public inquiry to address systemic issues in order to prevent the recurrence of such tragedies. Between November 2007 and February 2008, the Commissioner heard from government officials, prosecution and defense counsel, police officers, judges, and law professors as well as the internationally recognized forensic pathologists who were involved in the review of the specific cases. Research papers on a variety of topics were received and policy roundtable discussions conducted. Dr. Smith himself also gave evidence regarding his efforts and explanations.

The investigation of suspicious deaths in children presents many complex challenges for all concerned, including the need for proper training and certification of the forensic pathologist, evolving and sometimes controversial issues in pediatric forensic pathology, the difficulty in choosing appropriate language to characterize the level of certainty of an opinion regarding cause of death and the dangers of "tunnel vision."

Evidence at the inquiry identified a variety of approaches to address these problems. The implementation of a comprehensive postmortem report format detailing all opinions and the basis for same ensures a standardized methodology for the timely communication of autopsy findings. A vigorous peer review process prior to the release of such reports contributes significantly in validating the conclusions. The principles of evidence-based medicine provide an important standard to implement and ensure the requisite degree of reliability for a court considering opinion evidence on issues involving pediatric forensic pathology. Appropriate measures of accountability are necessary to identify and deal with those circumstances in which the pathologist's practices may be deficient. Continuing education for counsel and

experts will assist in avoiding misunderstanding of their respective roles and participation in the trial process.

While the inquiry received considerable publicity in Canada, the lessons which may be learned from these unfortunate events should be shared with the international forensic community in order to achieve the objectives of fairness and justice for those charged with criminal offences.

**Forensic, Pediatric, Pathology**

## G47 The Possibilities and Limitations of Neuropathology in Exhumation Autopsies

*Jan E. Leestma, MD\*, 1440 North Kingbury Street, Suite 210, Chicago, IL 60622*

After attending this presentation, attendees will understand important opportunities in difficult situations which commonly surround exhumation autopsies.

It is often assumed that exhumation autopsies will yield only marginal information, least of all to permit in-depth neuropathological examinations. This presentation will impact the forensic science community by demonstrating how five cases prove otherwise. This information should be valuable to pathologists and the general forensic community,

Five cases are presented that offer a range of forensic/medical issues in the context of exhumation autopsies. Four cases involved civil or criminal litigations and one case was done for personal reasons of a family of a suicide victim.

The durations of burial ranged from two to more than 18 years. All individuals had been interred in either wooden or metal caskets, usually within concrete burial vaults. All had been arterially embalmed. At issue were the nature of the injuries or processes leading to death that usually involved head trauma, but in one case involved possible Marchiafava-Bignami syndrome.

Three of the cases had not been autopsied before, but two had. In general, preservation of the head, brain, and other organs was good to excellent and permitted satisfactory case analysis both grossly and microscopically.

The opportunities and limitations of exhumation autopsy neuropathology will be presented.

**Exhumation, Neuropathology, Autopsy**

## G48 Pathology/Odontology: The Team Approach to a Forensic Autopsy

*John E. Filippi, DDS\*, 1325 North 127th Avenue, Omaha, NE 68154; and Mary H. Dudley, MD\*, Jackson County Medical Examiner's Office, 660 East 24th Street, Kansas City, MO 64108*

After attending this presenation the attendees will undertand the "dual role" the pathologist and odontologist have in determining the cause of death and the development of a positive identification

The presentation will impact the forensic community by increasing the awareness of the medical legal system and law enforcement agencies regarding how the coordination of these two forensic sciences can support the investigation.

A forensic pathologist performs autopsies to determine the cause and manner of death in situations falling under the jurisdiction of the Medical Examiner/Coroner Office. After the forensic autopsy is completed, the forensic odontologist examines the dental structures, and through a comparison analysis between the antemortem dental and the postmortem dental records, can render a "rapid onset" positive identification. When working together, both forensic professionals, can

provide concordance, to the issuing of a prompt death certificate for the next of kin, and can also be called upon to be an expert witness in a court of law.

This presentation will increase the awareness of the forensic community and law enforcement agencies, in the attempt to show, how both fields can work together in the forensic autopsy. The focus of this case presentation will highlight the forensic investigation of a high profile dual homicide case illustrating the forensic team approach. The difficulty of the case stems from the young age of the two related victims, the cause and manner of death, the history behind that investigation, and the final court decision. The presentation will include how forensic anthropologists recovered the buried remains, how forensic pathologists determined the cause and manner of death, and how forensic odontologists determined the chronological dental age and the final rendering of a positive identification.

Skeletal remains were discovered in a shallow grave in a wooded area in Missouri. Forensic Investigators first surveyed and photographed the scene. A forensic anthropologist was then called to assist with the excavation. Two juvenile skeletal remains along with clothing fragments and projectiles were placed into evidence. The forensic pathologist determined that both children were shot in the back of the head, from an indeterminate range, which was the cause of death. The skeletal remains were further examined by the forensic anthropologist, who determined estimates of height, weight, and race. After procuring antemortem dental records, a positive identification was made by the forensic odontologist. In addition, bone samples were collected and stored for DNA analysis.

Discussion of the dental (oral) autopsy will reveal how the actual dental x-ray comparisons were made for a positive identification. Specific reference will be made to the use of special dental technology, such as digital dental radiographs and the WINID dental charting software program. These same dental protocols can also be developed in the SOP's and applied by a medical examiner/coroner's office, for future multiple deaths or a mass fatality incident.

Although this was a high profile homicide case, the routine utilization of a forensic odontologist can provide additional evidentiary value to many cases involving skeletal, decomposed, or fragmented remains. Forensic dentistry can provide support evidence for positive identification, when other modalities, such as fingerprints or time sensitive DNA analysis are not utilized.

In conclusion, the ability to blend the two forensic sciences, pathology and odontology, during a forensic autopsy, can be invaluable to a medical examiner/coroner office in the investigation process, criminal trial and for final closure for the victim's families.

**Pathology, Odontology, Positive Identification**

## G49 Making the Best of Death

*Chantal Ferraro, PhD\*, Long Island University, Sociology/Anthropology, CW Post, Brookville, NY 11548; and O. C. Smith, MD, Conscience and Science in Medicine, Atoka, TN 38004*

The goals of this presentation are to: (1) evaluate the existing notion of social autopsy, (2) modify and expand it both conceptually and methodologically, and (3) integrate findings of physical and social autopsies in order to establish a bridge between the forensics of the dead and the forensics of the living.

This presentation will impact the forensic community by providing a model that is applicable to any population in any community, the understanding of which will make the study of the dead a powerful tool of death prevention for the living.

The term autopsy most commonly refers to the postmortem examination of a deceased for medical or forensic reasons. In that context, autopsy is synonymous with necropsy. Etymologically, however, autopsy translates into "to see for oneself." Recently, the addition of modifiers, such as social or psychological, to the term reflects

the attempt to define new fields of inquiry based on the broader latter meaning. A psychological autopsy examines the mental state of a deceased at time of death, and may contribute to the determination of the manner of death, especially in cases of suicide. The concept of social autopsy is more confusing since its two applications belong to entirely different spheres. According to Rick Lavoie (2005) a social autopsy is a pedagogical strategy to help a learning disabled child to see for himself the cause/effect relationship between his social behavior and the reactions of others. But for sociologist Eric Klinenberg, who studied the 1995 lethal heat wave in Chicago, a social autopsy is a way to identify the social risks of a dependent population factors that led the isolated, the old and the poor to die by the hundreds. Although valid and useful in their own right, these definitions are not adequate for us.

The model of social autopsy being discussed originates in the longitudinal study of child death which was presented at the 2002 AAFS meeting. The original study was based on the autopsy reports of the Shelby County (TN) Medical Examiner's office where over 1,500 cases of child death were investigated, inclusive of all manners, over a period of ten years. The "Swiss Cheese" concept elicited by the Human Factors Analysis and Classification System (HFACS) was incorporated where defects in the layers of latent and active responsibilities, permit lethal latent and active failures within individuals, society and cultures to be identified. This presentation will focus on homicidal deaths from 0 to 4 years of age and accidental deaths from 0 to 18. Subsequent sociological and anthropological research of the community in which these deaths occurred allowed us to "see for oneself" the levels of failure and the failures within levels. From this evolves an understanding of what produces not just the death of each individual but identifies patterns that anticipate future trauma or death, with its human and societal costs. These patterns represent a framework of behaviors that must be altered or remedied by the community. It is the contention of the investigators that a social autopsy is defined by its ability to reveal such patterns and to highlight the line of failure. The necropsy exists to identify this "tip of this iceberg" the social autopsy defines its magnitude.

**Social Autopsy, Necropsy, Prevention**

## G50 Building the Communication/Language for Collaboration Between the Forensic Pathologist and Funeral Director/Embalmer

*Vincent E. Hill, MD*, Mortuary Medical Services, 3003 Van Ness Street, North West, Suite 106, Washington, DC 20008*

After attending this presentation, attendees will be familiar with the language and techniques used in the funeral industry.

This presentation will impact the forensic community by arming the forensic pathologist with the words to scientifically describe a body that has been embalmed.

This presentation will expose the forensic pathologist to the language and embalming procedures used in preserving the dead body. The presentation will be useful to forensic pathologists who are members of FEMA's Disaster Mortuary Operational Response Team (DMORT) or work in jurisdictions where cemeteries are subject to flood waters.

Forensic pathologists are known for their skill in turning the visual into words. They are able to explain orally and in writing the most complex surgical procedures despite the fact that they are not trauma surgeons, orthopedic surgeons, neurosurgeons or gynecologists. But, when it comes to the postmortem surgical procedure known as embalming, the forensic pathologist lacks knowledge of the words and technical procedures used by the funeral director/embalmer. Michael M. Baden, MD mentions, in Chapter III, Part 4, "Exhumation" (Medicolegal Investigation of Death, 4th edition), "The entire exhumation and autopsy

process should be well documented by words and photographs …". As stated above, forensic pathologists know the words of their fellow surgeons but are not trained in the words of the funeral director/embalmer despite the fact that autopsies are performed on embalmed bodies prior to interment and after disinterment. Since the conception of DMORT in the early 1980's, national forensic teams have been deployed to 24 mass disaster events. Three of these events exclusively involved cemetery floods, one involved a crematory, and three involved floods that secondarily caused local flooding of cemeteries. With disinterred remains the primary focus of the forensic team is not in determining the cause of death but in finding positive identification. With disinterred embalmed bodies, the presence of embalming artifacts may be one of the main physical findings the DMORT forensic pathologist will have for positive identification. If the forensic pathologist is unable to adequately describe the postmortem embalming changes that occur during arterial and cavity embalming he or she could exclude or misinterpret a useful identifier. If the forensic pathologist knows that the body was embalmed using a single-point injection through the right common carotid artery with right jugular vein drainage or was embalmed by use of the restricted cervical method, this level of knowledge would help expedite the identification process when the local funeral director/embalmer compares their embalming reports with the autopsy report. Since 29% of DMORT's mass fatality events involve disinterred remains, the affected community would be better served by having forensic pathologists who are able to speak and write the language of the local funeral director/embalmer.

**Forensic, Pathologist, Embalming**

## G51 Sickle Cell Trait Associated Deaths: A Case Series With a Spectrum of Clinical Presentations

*Christopher Wilson, MBBS, Jon R. Thogmartin, MD*, Noel A. Palma, MD, and Susan S. Ignacio, MD, Medical Examiner's Office, District 6, 10900 Ulmerton Road, Largo, FL 33778; Barbara C. Wolf, MD, and Wendy A. Lavezzi, MD, Office of the Medical Examiner, District 5, 809 Pine Street, Leesburg, FL 34748; and Mark J. Shuman, MD, Miami Dade County, Medical Examiner Department, Number One on Bob Hope Road, Miami, FL 33136*

After attending this presentation, attendees will be educated with respect to the wide variety of clinical presentations persons with sickle cell trait manifest including clinical symptoms, laboratory abnormalities, and gross anatomic and microscopic findings. This study also seeks to show how simply the diagnosis can be made by the astute clinician or forensic pathologist if only he or she will consider it in the differential diagnosis.

This presentation will impact the forensic community by showing that sickle cell trait is a condition which is not restricted to conventional ethnic boundaries of Afro-Americans and that the diagnosis needs to be seriously considered in individuals living in geographic locations in which the natural environment plays a prominent role in the manifestation of the disease. Early recognition of the disease in such individuals can possibly result in a decline in mortality.

At the conclusion of this presentation, attendees will be educated with respect to the wide variety of clinical presentations persons with sickle cell trait manifest including clinical symptoms, laboratory abnormalities, gross anatomic and microscopic findings. This study also seeks to show how simply the diagnosis can be made by the astute clinician or forensic pathologist if only he or she will consider it.

This presentation will hopefully have a significant impact on not only the forensic community but also on humanity by showing that sickle cell trait is a condition which is not restricted to conventional ethnic boundaries of Afro-Americans and that the diagnosis needs to be

seriously considered in individuals living in geographic locations in which the natural environment plays a prominent role in the manifestation of the disease. Early recognition of the disease in such individuals can possibly result in a decline in mortality.

As many as one in three Africans living in areas where malaria is indigenous and approximately one in twelve Americans with African ancestry have sickle cell trait. The affected individuals are generally asymptomatic and many are not even aware that they carry the gene. The general consensus of the public is that sickle cell trait is a relatively benign condition and affected persons are at no increased risk of morbidity or mortality because of their condition. However, the forensic community is cognizant that under the proper set of circumstances, sickle cell trait can be potentially fatal.

This study presents a series of 11 individuals with sickle cell trait and one with hemoglobin SC disease who died during various circumstances. All of the victims were subject to the warm and humid climate of Florida. The onset and/or duration of symptoms varied from a few to several hours with many displaying a prolonged lucid interval with stable vital signs. Despite seeking medical treatment, sickle cell trait related micro-occlusive crisis was never considered in the differential diagnosis. Several cases were associated with sudden death. In those deaths which were delayed, high anion gap and uncompensated metabolic acidosis were typical. Also characteristic were large increases in creatine phosphokinase, alanine aminotransferase and aspartate aminotransferase along with myoglobinemia. Although the antemortem diagnosis of rhabdomyolysis was made, the underlying cause was never deduced by the clinicians. Of particular interest was a case of a fatal splenic crisis due to sickle cell trait in a Caucasian and a victim with hemoglobin SC who died from a combination of mild traumatic injuries and prolonged bodily inversion. In some cases, sickle cell trait was not even considered in the original death certification.

In conclusion, this study demonstrates the varying characteristics and presentations of 11 cases of sickle cell trait and one case of hemoglobin SC related deaths and shows that such deaths can be sudden or delayed. Conventional racial delineation of the sickle cell hemoglobinopathies should not deter one from considering it in the differential diagnosis especially if the patient is subjected to environmental and physical stressors which can potentiate the disease. Furthermore, failure to consider sickle cell trait related crises as a diagnosis can result in improper death certification. Greater efforts to educate the public especially athletes and coaches on the possible hazards of exercise induced sickle cell trait related micro-occlusive crisis hopefully will result in less morbidity and mortality.

**Sickle Cell Trait, Exertion, Metabolic Acidosis**

## G52    Commotio Cordis: A Forensic Science Perspective

*Ariel Goldschmidt, MD\*, Steven T. Hensley, and Mary H. Dudley, MD, Jackson County Medical Examiner's Office, 660 East 24th Street, Kansas City, MO 64108*

After attending this presentation, attendees will have an enhanced understanding of the prevalence, pathophysiology, and important forensic science issues related to commotio cordis and the ability to apply this knowledge to their practice should it become necessary.

This presentation will impact the forensic community by providing a broad and thorough review of the current literature and scientific knowledge about commotio cordis, with particular emphasis on key issues relevant to forensic scientists. Included in the discussion will be several published case examples of commotio cordis, as well as several case examples of commotio cordis investigated in the State of Missouri.

The presentation will begin with detailed criteria for what type of deaths do and do not constitute commotio cordis. Mention will be made of the prevalence of commotio cordis and common involved activities, with specific published case examples involving sports activity and a low speed vehicle collision. Case reports resulting from a retrospective search of cases in Missouri will be presented.

Attention will next be focused on animal models used for the study of the pathophysiology of commotio cordis. The theory of "mechano-electric coupling" of myocyte stretching and the opening of potassium/ATP channels will be discussed. Lessons learned from various experiments using animal models will be presented, including the importance of hardness of sports objects and effectiveness of chest protectors.

The presentation will include several issues of particular interest to medicolegal investigators and forensic pathologists. Typical as well as atypical case histories of commotio cordis will be presented, stressing the importance of proper scene investigation and obtaining medical history. There will be a review of possible autopsy findings seen in cases of commotio cordis. Finally, controversies regarding manner of death in atypical cases of commotio cordis will be discussed.

**Commotio Cordis, Precordium, Manner of Death**

## G53    Corpora Amylacea and Sudden Death: A Case of Adult Polyglucosan Body Disease Diagnosed at Forensic Autopsy

*Timothy L. Williams, MD\*, and R. Ross Reichard, MD, New Mexico Office of the Medical Investigator, MSC11 6030, 1 University of New Mexico, Albuquerque, NM 87131-0001*

After attending this presentation, attendees will learn about a case of adult polyglucosan body disease diagnosed at forensic autopsy.

This presentation will impact the forensic community by educating them about the first reported case of adult polyglucosan body disease presenting as sudden death and diagnosed at forensic autopsy.

Adult polyglucosan body disease (APBD) is a rare neurodegenerative condition characterized by typical onset in middle age, progressive neurological impairment that is heterogeneous between those affected, and death within 1-14 years of diagnosis. The histopathological hallmark of the disease is massive deposition of corpora amylacea (designated polyglucosan bodies in this context) in the central nervous system, and variable deposition of similar material in other sites. While the cause of the disease is as yet unknown, recent research has identified mutations in proteins involved in glycogen metabolism in a subset of cases. Some of these mutations are similar to mutations identified in cases of glycogen storage disease type IV (GSD IV), a disease that classically is present in the first year of life, is of very heterogeneous manifestation, and is also characterized by massive deposition of corpora amylacea. The genetic and histopathological similarities between these two conditions have lead to speculations that APBD may represent an adult form of GSD IV.

In this presentation, a case of sudden death is presented wherein APBD was diagnosed at forensic autopsy. Scene details, relevant medical and social history, and autopsy and histopathological findings are presented and richly illustrated with supporting images.

The case provides an excellent example of a prolonged and enigmatic presentation involving a complex interplay of medical, social, and forensic issues. The histopathology is particularly illustrative of this rare disease, showing massive deposition of corpora amylacea in the central nervous system, and marked accumulation of similar material in the heart. The latter was determined to be the mechanism of death (cardiac arrhythmia) with APBD the underlying cause.

This presentation represents the first case of APBD reported in a forensic context. APBD is reviewed and its relationship with other

diseases characterized by massive deposition of corpora amylacea is outlined. The role of forensic autopsies in the diagnosis of rare conditions is discussed.

**Neuropathology, Corpora Amylacea, Sudden Death**

## G54　Trends in Forensic Investigations Into the Missing:　Observations From the ICRC

*Morris Tidball-Binz, MD\*, Ute Hofmeister, MA, and Shuala M. Drawdy, MA, International Committee of the Red Cross, 19 Avenue de la Paix, Geneva, 1202, SWITZERLAND*

After attending this presentation, attendees will gain awareness of trends identified in the application of forensic medical sciences to investigations into the whereabouts and fate of persons missing as a result of armed conflict, internal violence, or catastrophes, as observed by the forensic unit of the International Committee of the Red Cross (ICRC). Attendees will also learn about the steps taken by the ICRC to meet challenges posed by these trends.

This presentation will impact the forensic community by outlining emerging challenges posed to the wider forensic community by investigations into persons gone missing as a result of armed conflict, internal violence or catastrophes, as observed by the ICRC. The trends and challenges identified in this paper will assist in the design of strategies for effective and efficient contribution to this emerging field by forensic practitioners, institutions, and service providers.

In February 2003, the ICRC organized an International Conference on The Missing in Geneva, Switzerland. Recommendations were adopted to prevent and resolve the tragedy of persons unaccounted for as a result of armed conflict and internal violence. These included recommendations on forensic best practices for the recovery, management and identification of the dead in challenging contexts, including: roles, duties, responsibilities and applicable ethical standards for forensic practitioners and teams; guidelines for the recovery and storage of human remains; criteria for forensic human identification; principles for ethical, effective and efficient information management; and advice on the relationship between forensic practitioners and bereaved families and communities.

Following the International Conference, the ICRC established a forensic unit to help implement the recommendations worldwide. Since its inception, the forensic unit has witnessed a sustained increase in the application of forensic medical sciences to the search for The Missing and has observed the following trends:

- Growing awareness and understanding of the tragedy of The Missing in armed conflicts and catastrophes;
- Growing recognition of the importance of proper management and identification of the dead in armed conflicts and catastrophes;
- Growing needs for experienced forensic practitioners for investigations into The Missing;
- Incorporation of recommendations from the 2003 International Conference on The Missing into international standards and national legal and institutional frameworks related to The Missing;
- Awareness of the need for sustainable local forensic capacity to investigate The Missing;
- A role-shift from medicolegal practitioners (i.e. coroner, medical examiner, forensic doctor) towards multidisciplinary forensic teams in the recovery and identification of The Missing;
- Growing reliance on forensic DNA analysis;
- Demands for professional standards of best practice and quality assurance and control from practitioners and institutions involved in investigations into The Missing;

- Increased incorporation of investigative practices regarding
- The Missing into scientific literature, research and training; and
- Growing expectations from bereaved families and the general public for swift and positive results from forensic investigations into The Missing.

These trends pose challenges and inherent opportunities for the forensic community, including:

- Helping to meet growing needs worldwide for forensic practitioners, institutions, and service providers for investigations into The Missing. These should conform to standards of professional best practice, quality assurance and control required for these investigations. Local capacity building and ownership should be prioritized;
- Empowering communication, coordination and cooperation, at regional and worldwide levels, between forensic practitioners, institutions and service providers involved in and available for investigations into The Missing;
- Supporting swift access to indispensable forensic know-how, technology and tools by practitioners and institutions operating in under-resourced contexts; and
- Sustained efforts in public awareness raising about the role, scope, value and limitations of forensic sciences applied to investigations into The Missing.

The clarification of the whereabouts and fate of The Missing in armed conflicts and catastrophes is a humanitarian priority that requires a global and concerted effort, including from the forensic community. Based on trends observed and lessons learned, the ICRC offers recommendations for addressing the challenges identified in this paper and also for building on the opportunities which these challenges offer to the forensic community.

**Missing Persons, Humanitarian Identifications, International Committee of the Red Cross**

## G55　Evidence-Based, Medical-Legal Documentation of the Postmortem Anogenital Examination

*Sharon R. Crowley, MN\*, FCNS, 122 Emeline Avenue, Santa Cruz, CA 95060*

After attending this presentation, attendees will understand how to incorporate an evidence-based methodology for the documentation of the postmortem genital examination. Attendees will also be able to facilitate incorporation of a previously proposed taxonomy, germane to the postmortem anogenital examination, in order to improve medical-legal documentation and able to incorporate a theoretical framework for sexual murders, as a basis for the methodological examination of the suspected sexual homicide victim.

This presentation will impact the forensic community by augmenting and enhancing the forensic examiner's diagnostic acumen in this arena. Helping to avoid ambiguity among examiners in the interpretation of clinical findings and improve documentation and ultimately contribute to a better understanding of the etiology and manifestations of fatal sexual violence against women.

The interpretation of genital findings in the deceased remains a vital and timely issue. Until recently, a paucity of information existed on the nature and appearance of the anogenital tissues during the postmortem interval. Because the traditional genital examination consists of gross visualization, subtle findings were not easily detected. These findings may constitute injury due to sexual assault, concomitant changes in the anatomy due to postmortem processes of decomposition, or a combination of both. The theoretical framework for this proposed methodological documentation format is founded on:

- Sexual activity by the offender that culminates in the death of the victim.
- Current, ongoing baseline clinical studies on the nature and appearance of the anogenital anatomy during the postmortem interval.
- Previously presented methodology for postmortem genital examinations (Crowley, JFS, 2004).
- Previously described taxonomy for the description and classification of the appearance of the tissues during the postmortem anogenital examination.

Currently, a wide variation exists in methodology for examination of both antemortem and postmortem sexual assault victims. Postmortem challenges vis-á-vis protocols and procedures may pose even greater significance, because there is no surviving victim to recount details of the assault, including sexual acts, threats, and other behaviors of the offender(s).

Currently, no standardized state, regional, or national form exists for the accurate and complete documentation of the postmortem anogenital examination. The clinical evaluation of the sexual homicide victim forms the basis for all related medical-legal reports.

- The question of exam authorization may be an area of concern. However, no separate authorization should be needed, as these examinations fall under the jurisdiction of the Coroner or Medical Examiner. In addition, they are medically non-invasive procedures.
- The postmortem genital examination record is not a complete medical record, as with other forms used to document the sexual examinations of *living* sexual assault victims, e.g., California Office of Emergency Services, forms 923 and 930. Therefore, supplemental medical and/or gynecological records may be of benefit for further review.

The meaning and performance of the acts committed during a sexual murder varies with the offender. Salient features of the crime may be evident, which may give information about the offender's sexual motivation. A systematic, evidence-based approach to documentation is part of a consistent, methodological approach to the evaluation of this population.

Scrupulous documentation should provide as much data as is known about a given case. This includes the following general categories: salient case and demographic data, disposition of the body, available history, general physical assessment, clothing, toxicology, evaluation of nongenital trauma, components of the sexual assault evidence kit, the genital and anal examination, and colposcopic examination. It is important to clarify where the primary responsibility for a portion of the examination and/or the documentation was *not* assumed, e.g., in the evaluation of nongenital trauma by a forensic nurse.

Select cases of fatal sexual violence provide actual examples of traumatic injuries, consistent with blunt force trauma to the anogenital tissues. Injuries can be categorized as to type, number, and anatomic site. In addition, normative studies of baseline controls were evaluated, using the sequential methodology for the postmortem genital examination with colposcopy, *SART-TO-GO* (Crowley, JFS, 2004). A previously presented taxonomy was developed to describe the nature and appearance of the postmortem genital anatomy (Crowley & Peterson, AAFS, 2004) and to develop a standardized classification system for these previously undescribed findings. This taxonomy is incorporated in the proposed protocol for the documentation of the medical-legal examination of sexual homicide victims.

The postmortem genital anatomy worksheet consists of the same anatomic sites that are routinely examined in the living sexual assault victim. These include the peri-urethra/peri-clitoral area, labia majora, labia minora, posterior fourchette, fossa navicularis, hymen, vagina, cervix, perineum, anus, and rectum. Supplemental documentation is included for both the adult male and the pre-pubertal child.

All examination techniques and any adjuncts should be recorded, such as use of balloon-covered swabs (Crowley, 1999), labial traction, or labial separation, in addition to routine speculum examination and anoscopy.

Appropriate and complete chain-of-custody must be documented and is included within the tool. A copy of the tool should be placed within the sexual assault evidence kit for the criminalist.

A supplemental narrated summary or dictation is recommended, to complement the standardized form. It also places events in chronological order, incorporates more uniformly understood language, and clarifies roles and responsibilities. The emphasis on teamwork and evaluation is crucial in an event that requires multi-disciplinary cooperation.

The taxonomy for postmortem genital examinations has proved to be a useful classification system during clinical examination, case documentation, and database entry. This taxonomy has been incorporated into the proposed medical-legal form, in order to differentiate postmortem artifact from concomitant findings that might be suggestive of sexual trauma, i.e., blunt force injury, including lacerations, ecchymoses, and abrasions. This capacity is pivotal. Just as in living victims, it is essential to be able to distinguish benign gynecological conditions from traumatic findings.

Data to date from the analysis of ongoing, baseline controls has yielded useful information for the development of a template for documentation. It is important to carefully describe the nature and appearance of salient anatomic sites. Analysis of research data has reinforced the need for the examiner to avoid working in a vacuum. The examiner whose sole prior experience lies in the antemortem arena may confuse normal postmortem artifact with traumatic findings. Common benign gynecological findings, such as labial adhesions and punctate lesions, are often present. Other findings, such as postmortem mucosal shedding at various sites within the anogenital tissues, have occurred with sufficient frequency during the postmortem control study to warrant recognition as normal postmortem artifact.

The ultimate goal is to better visualize and improve the understanding of what is normal in anogenital anatomy during the postmortem interval. To this end, careful scrutiny and meticulous documentation will both add to individual case yield and enhance the overall body of knowledge within this area.

**Postmortem Anogenital Examination, Sexual Homicide, Colposcopy**

## G56    Postmortem Recognition of Sickle Cell Trait

*Kathryn H. Haden-Pinneri, MD\*, and Sara Nunez-Doyle, MD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will gain a better understanding of the importance of recognizing the incidental autopsy findings related to sickle cell trait and the implications this diagnosis may have on surviving family members.

This presentation will impact the forensic community through knowledge gained about sickle cell trait and from insight regarding the importance of notification of surviving family members.

Sickle cell trait is defined as the heterozygous condition of having one gene for sickle cell hemoglobin and one for normal hemoglobin. Patients with sickle cell trait tend to lead normal lives, without serious complications; therefore it is not regarded as a disease state. In certain conditions; however, complications not only occur, but they may be fatal. Deaths due to exertional sickling involving young athletes have made headline news on multiple occasions. When an individual with sickle cell trait becomes hypoxic, acidotic, dehydrated, or hypothermic, the typically silent sickle cell trait transforms into a syndrome that resembles sickle cell disease with widespread sickling and subsequent vaso-occlusion.

The presence or absence of intravascular sickled red blood cells in tissue specimens depends on the degree of oxygenation of the sample

prior to fixation. Intravascular sickling may occur due to terminal hypoxemia in the setting of sickle cell trait. It is almost impossible to determine the role sickled cells may have played by the presence or absence of intravascular sickling in autopsy specimens.

The events surrounding a death due to exertional sickling will assist the pathologist in this diagnosis. Individuals with sickle cell trait who die without a history of intense exercise prior to death may pose a challenge to physicians in determining if the death is or is not related to their genetic condition. A third possibility exists in which an individual may not be known to carry the sickle cell trait until sickled cells are seen in biopsy or autopsy specimens.

Three decedents autopsied at the Harris County Medical Examiner's Office, ranging in age from 28 to 49 were found to have sickle cell trait. None of the individuals were known to have the trait and when family members were contacted, only one had any knowledge that this condition existed in their family. The sickle cell trait was found to be purely incidental in two of the three decedents and may or may not be related to the cause of death in the third individual.

Two of the decedents were black males who were found unresponsive at work, one outside and one at a desk. One was 39-years-old and the other was 49-years-old. Both had enlarged hearts with coronary artery atherosclerosis. Microscopic examination revealed sickled cells in the heart, liver, lungs, kidney, and brain of both men. Hemoglobin electrophoresis performed on postmortem blood revealed the presence of hemoglobins A, S, F, and A2 in levels suggestive of sickle cell trait with an underlying beta+ thalassemia.

The third case involved a 28-year-old morbidly obese black female (body mass index of 54.1) who became unresponsive shortly after complaining of shortness of breath and abdominal pain. Autopsy findings included bilateral pulmonary thromboemboli, deep venous thromboses, gallstones, and clear bile. Microscopic examination revealed sickled cells in the kidney liver and brain. Hemoglobin electrophoresis results are pending at this time. Family members were contacted and reported knowledge of sickle cell trait in a sibling, but not in the decedent.

All family members contacted were grateful for the information and most were planning a follow up visit with their physician to obtain testing for sickle cell trait. With the exception of possibly the pulmonary emboli, the finding of sickle cell trait was incidental to the determination of the cause and manner of death; however, the information was extremely important for the family members of the decedents. This finding underscores the responsibility of forensic pathologists to perform autopsies with the intent of complete and thorough documentation of all findings, not just determination of cause and manner of death.

**Autopsy, Sickle Cell Trait, Incidental**

## G57 An Angel Dies on the Needle: Fatality After Injection Sclerotherapy for Prolapse Rectum in a Child

*Abraham T. Philip, MD\*, Onondaga County Medical Examiner, 100 Elizabeth Blackwell Street, Syracuse, NY 13210; and Jeanna M. Marraffa, PharmD, Upstate New York Poison Center, 250 Harrison Street, Syracuse, NY 1302*

After attending this presentation, attendees will be made aware of the toxicity of phenol, a product with multiple clinical applications that resulted in a fatality during a non-invasive surgical procedure.

This presentation will impact the forensic community by increasing its awareness about a previously unreported complication of use of phenol as a sclerotherapeutic agent for prolapse of rectum.

The goal of this presentation is to discuss the findings in a case of toxicity due to phenol toxicity that resulted in the death of child fatality after a surgical procedure for a non-life threatening condition.

Prolapse rectum (PR) or protrusion of the rectum beyond the anus occurs frequently in populations at both extremes of age. In the pediatric population, PR is usually diagnosed before the age of three years, and in adults, the peak incidence of PR is after the fifth decade of life. The etiology of PR in developing countries is usually related to diarrheal illnesses, parasitic infestations, and malnutrition. In the developed world, a common cause is cystic fibrosis. Surgeons have shown considerable ingenuity in the search for the ideal operation for PR. Over 200 different procedures have been employed, suggesting that the ideal surgical solution has remained elusive. Treatments include conservative management, resection and fixation, levator ani repair, presacral packing, Thiersch's wire suture and injection sclerotherapy. The last is considered an attractive treatment option because it is minimally invasive.

The case presented is of a 2-year-old female child, with PR, cystic fibrosis, and Ebstein malformation of the tricuspid valve. Due to refractory PR, the decision was made to treat her with injection sclerotherapy, using phenol as the sclerotherapeutic agent. In the operating room, and shortly after the injections, the baby had a sudden cardiac arrest, and received CPR for approximately 2 hours. She developed anoxic encephalopathy, rhabdomyolysis, non-hyperthermic elevated creatine kinase (CK) levels, and disseminated intravascular coagulopathy (DIC). She died approximately 4 days after the surgery. An antemortem urine specimen submitted on the day after the surgery had a total phenol concentration of 240mg/L. Phenol concentrations, done as part of workplace testing, in unexposed and chronically individuals should be below 10 and 30 mg/L.

At autopsy, the baby's external appearance and internal organs were appropriate for age. There was a reddish discoloration around the anus, and separate reddish brown discoloration to the buttocks. Internally there were multiple punctuate hemorrhages on the mesentery and capsule of internal organs. There was intraparenchymal hemorrhage within the lungs and spleen, and red blood cell casts within the renal pelvis. The anal canal was infarcted. The evaluation of the heart (48.2 grams) confirmed the Ebstein malformation. The muscle biopsy revealed nonspecific congenital myopathic changes with decreased myophosphorylase and glycogen. The section of liver showed PAS & PASD negative vacuolization of the hepatocytes. Examination of the brain revealed cerebral edema with acute hemorrhagic infarct of the left occipital cortex, and multifocal subarachnoid, cortical, and cerebellar hemorrhages.

A variety of sclerosing agents have been used with varying success rates. Phenol preparations have been used in dermatology and plastic surgery for the treatment of acne and during chemical face peels. During cutaneous application of phenol, absorption of the chemical has occurred with deleterious systemic effects, including cardiac arrhythmias have been reported. The publications about the value of the use of Phenol as sclerosing agent for PR have been mixed. One report indicated 90 to 100% cure rates after one or two injections and no complications. Another report indicated complications, including mucosal sloughing and perianal fistulae, in 27% of cases. No cases of fatality due to phenol toxicity after injection sclerotherapy have been reported in the medical literature.

This case report describes the steps taken to establish the diagnosis of phenol toxicity, and eliminate the other causes of sudden death suggested by the initial differential diagnosis. The forensic community should be aware of the toxicity of phenol as it has multiple clinical uses, and can result in fatality.

**Prolapse Rectum, Injection Sclerotherapy, Phenol Toxicity**

## G58 MDMA Neurotoxicity

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After attending this presentation, attendees will gain knowledge on how to characterize MDMA neurotoxicity in rat brain.

This presentation will impact the forensic community by demonstrating how the results of even one single administration of MDMA can significantly alter the brains cellular antioxidant defense system and produce oxidative stress in both the striatum, and frontal cortex. Thus one possible mechanism of MDMA neurotoxicity appears to be a direct toxic effect of MDMA or its red-ox active metabolites

3, 4-Methylenedioxymethamphetamine (MDMA or "Ecstasy") is an increasingly popular psychoactive and hallucinogenic drug of abuse. It acts on the CNS by increasing the release of serotonin and other catecholamines in addition to preventing their reuptake. MDMA has been shown, in both man and animal, to damage serotonergic and dopaminergic nerve terminals and to cause neurodegeneration in multiple areas of the brain, including the cortex, hippocampus, striatum and thalamus. The closely related drug, methamphetamine (METH) and its derivates have been shown to produce long-lasting depletion in dopamine and its metabolites, as well as dopamine reuptake sites in the rat and primate striatum, but not in other dopamine rich areas such as the nucleus accumbens and the prefrontal cortex, in contrast to the neurotoxic effect of dopamine to striatal DA terminals. Two other important aspects of MDMA neurotoxicity have been identified: hyperthermia and neurodegeneration. The former appears to be a direct action of MDMA, while the later is due to the production of reactive oxygen (ROS). Mounting evidence suggests that MDMA-induced 5HT neurotoxicity is due to the increased production of free radical induced oxidative stress. Attempts were made to clarify the mechanisms of MDMA in rats' brain by administering a single dose of the drug and studying the effects using combined toxicological, biochemical and immunohistochemical analysis.

Fifty rats were used for the study, each weighing 200-250 grams. Twenty-five rats were used for the histopathological and toxicological examination. They were divided into three experimental groups of seven animals each and administered one 20mg/Kg dose of MDMA intraperitoneally. The four controls were injected with saline. The first group of animals was sacrificed six hours after injection, the second at 16 hours, and the third at 24 hours. Plasma samples obtained immediately after sacrificed, stored at – 80°C and then analyzed for MDMA/MDA with gas chromatography/mass spectroscopy (GC-MS). Histological sections of the brains were also obtained and immunohistochemical stains were used to localized MDMA and its metabolites, MDA and MDEA, within the various areas of the brain. Other immunohistochemical stains were used to localized growth associated protein 43 (GAP43}, tryptophan hydroxylase (TrypH), markers of synaptic plasticity of the serotonergic innervation and the vesicular monoamine transporter -2 (VMAT2) as a stable marker of striatal dopaminergic terminal integrity. Microglial activation and damage were measured using a different immunohistochemical stain for glial fibrillary acid protein (GFAP), Heat shock proteins (Hsp 27, Hsp 70 and Hsp 90) and beta-amyloid precursor protein ($\beta$APP). Apoptosis was measured using the tunnel assay, which identified apoptosis via DNA fragmentation. For the evaluation of oxidative stress, the others twenty-five rats, divided into three groups of seven male albino rats weighing 200-250 g were used to analyze the effect of MDMA administration (20 mg/Kg, i.p) on rats' brain. Hippocampus, striatum, and frontal cortex were removed 3 and 6 hours after treatment and analyzed for the activity

of antioxidant enzymes (super oxide dismutase, SOD; glutathione reductase, GR; glutathione peroxidase, GPx). Reduced and oxidized glutathione (GSH and GSSG) were measured using a spectrophotometric assay. Ascorbic acid (AA) levels were determined reverse-phase HPLC method. Finally, lipid peroxidation was measured by quantitating the release of malonaldehyde (MDA) using UV-HPLC.

The acute administration of MDMA produces a decrease of GSH/GSSG ratio and oxidative stress in all of the brain areas examined. SOD activity was significantly reduced after 3 hours in hippocampus (-60.7%) and after 6 hours in striatum, hippocampus, and frontal cortex (-43.3%, -86.1% and -23.4% respectively). GR and GPx activities were reduced after 3 hours (-22%) and after 6 hours (-33.3%) in frontal cortex. AA levels strongly increased in striatum, hippocampus and frontal cortex after 3 (+159%, +84% and 17.6%) and 6 (+162%, +154% and +23.4%) hours respectively. High levels of MDA respect to control were measured in striatum after 3 hours (+276%) and 6 hours (162%); in hippocampus (71.8%) and in frontal cortex (+18.22%) after 6 hours.

The results of even one single administration of MDMA can significantly alter the brains cellular antioxidant defence system and produce oxidative stress in both the striatum and frontal cortex. Thus one possible mechanism of MDMA neurotoxicity appears to be a direct toxic effect of MDMA or its redox active metabolites.

**MDMA Neurotoxicity, Immunohistochemical, Oxidative Stress**

## G59 Levorphanol, Dextromethorphan, and a Case of (Probable) Mistaken Identity

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After attending this presentation, attendees will recognize that levorphanol and dextrorphan, a metabolite of dextromethorphan, are stereoisomers that cannot be distinguished from each other by routine toxicology testing, and that forensic pathologists and others in receipt of toxicology reports should be cognizant of this when interpreting toxicology results.

This presentation will impact the forensic community by raising awareness among both forensic pathologists and toxicologists of the laboratory's limitations regarding levorphanol and dextrorphan discrimination, thereby leading to improved communication between pathologists and the laboratory along with a reduction in instances of misinterpreted toxicology results involving these compounds.

Appropriate evaluation of toxicology results within the context of a forensic autopsy is vital, and relies, in part, on a laboratory's ability to detect, differentiate, and report individual compounds contained within specimens collected during a postmortem examination. The existence of pharmacologically active stereoisomers poses an additional challenge to both toxicologists and pathologists, as they cannot be differentiated in the laboratory by routine methods. This is the case with levorphanol, a relatively potent prescription narcotic, and dextrorphan, the active metabolite of the commonly used over-the-counter antitussive dextromethorphan.

A case involving a 70-year-old man with pneumonia and a history of chronic ethanol abuse is presented to illustrate the importance of recognizing the laboratory's general inability to differentiate levorphanol from dextrorphan. Laboratory testing in this case showed a relatively high level of levorphanol along with other medications commonly found in over-the-counter cold medications. The presence of levorphanol was unexpected within the context of the case, as the decedent was taking no prescription medications and had not seen a physician for years. The

levorphanol was initially considered a significant contributing factor in the man's death.

Re-evaluation of the toxicology findings, spurred by a second case with similar toxicology results under equally incongruous circumstances, uncovered the difficulty posed to toxicology testing by the structural similarity between levorphanol and dextrorphan. Given this insight, the circumstances of both of these cases suggested that the compound originally reported to be levorphanol was considered more likely to be the metabolite of dextromethorphan. Subsequently, "levorphanol intoxication" was discounted as a factor contributing to death in the first case. The original toxicology reports were amended to reflect the inability to distinguish between levorphanol and dextrorphan.

Subsequent review of in-house case files since 1999 revealed 13 more cases in which levorphanol was reported to be in blood and/or urine along with other compounds often admixed with dextromethorphan in over-the-counter cold medications. These findings suggest that some, if not all, of these earlier cases were more likely to represent the detection of dextrorphan and not levorphanol.

Toxicologists and pathologists should be aware that levorphanol and dextromethorphan's metabolite dextrorphan are stereoisomers, and that their structural similarity renders them indistinguishable by routine laboratory testing. An understanding of these limitations is critical to the interpretation of toxicology results that may indicate the presence of one or both of these compounds.

**Levorphanol, Dextromethorphan, Isomers**

## G60    Fentanyl-Related Drug Deaths in Virginia (2000-2006)

*William T. Gormley, PhD\*, Office of the Chief Medical Examiner, 400 East Jackson Street, Richmond, VA 23219; and Anna Noller, PhD, Virginia Department of Health, Office of the Chief Medical Examiner, 400 East Jackson Street, Richmond, VA 23219*

After attending this presentation, attendees will recognize the growing contribution of fentanyl to drug deaths, especially in combination with other drugs and alcohol.

This presentation will impact forensic scientists, primary care physicians, pharmacists, and patients by demonstrating the danger of combining fentanyl with other medications, street drugs, or alcohols.

Fentanyl is an opiate analgesic with about 81 times the therapeutic effect of morphine. Initially used mostly in hospital settings, fentanyl is increasingly used for outpatient management of chronic pain, especially delivered transdermally or through mucous membranes. Over the past years fentanyl has appeared more frequently in toxicology screens associated with drug deaths.

All deaths investigated by the Virginia Office of the Chief Medical Examiner between January 1, 2000 and December 31, 2006 in which fentanyl was detected on toxicological examination were received. Cases where fentanyl was used therapeutically in natural deaths were excluded from this analysis. Cases where fentanyl was present but the cause of death was a traumatic injury were excluded.

Analysis demonstrated a progressive increase in number of cases from three in 2000 to 51 in 2006. Deaths involving fentanyl typically occurred in the 3rd and 4th decade of life (average 40 years). There was a slight male predominance (about 60%), and 97% of the victims were white. There was significant geographic disparity in the data. There are four District Offices in Virginia, each office serving approximately 25% of the population. The relatively rural Western District had 51% of the fentanyl-associated death cases. The more urban Central, Eastern, and Northern Districts had 15%, 19%, and 15% of the cases respectively. Most of the deaths were classified as accidental (88%) with 10% suicidal and 2% undetermined.

Only 12% of the deaths in this study were caused by fentanyl alone. In the remaining cases other drugs were present and contributed to the death. The other drugs included prescription medications, street drugs, over the counter medications, and alcohol. Prescription medications were involved in 85% of the cases and included analgesics, muscle relaxants, and mental maintenance drugs. Prescription drugs were over-estimated in this study since medications which may have been obtained illegally (i.e., oxycodone and methadone) were classified as prescription drugs. Morphine was classified as prescription unless 6-acetylmorphine was also present. Street drugs were involved in 14% of cases, over-the-counter drugs (acetaminophen, antihistamines, and dextromethorphan) in 8% and alcohol in 13%.

This analysis documents the marked increase (17-fold) increase in fentanyl-related deaths over the last six years. The observation that most of the deaths are associated with other drugs suggests a role for increased caution by physicians in prescribing fentanyl, especially in combination with other medications. Increased education of patients is essential with emphasis on the critical importance of using the medication as directed. Patients should also understand the danger of mixing fentanyl with non-prescribed substances such as street drugs, alcohol, and sedating over-the-counter drugs.

**Fentanyl, Drug Death, Epidemiology**

## G61    Deaths Involving Stress

*Jeffery J. Gofton, MD, 901 North Stonewall, Oklahoma City, OK 73117-1218; and Wendy M. Gunther, MD\*, Office of the Chief Medical Examiner, Tidewater District, 830 Southampton Avenue, Suite 100, Norfolk, VA 23510-1046*

After attending this presentation, attendees will learn characteristics of traumatic and natural lesions identified in a series of cases presenting for medicolegal autopsy in which the medical examiner determined that stress played a role in death; will be able to evaluate the role of toxicology, history, and time course in determining the cause and manner of death in such cases; and will be able to evaluate impact of such characterization of cause and manner of death on courtroom testimony.

This presentation will impact the forensic community by demonstrating how to utilize a case format series to evaluate the common factors which should be present in order to assign stress a role in certification of medicolegal death. Discuss the appropriate manner in each of several cases of stress-related death.

Although most deaths presenting for medicolegal autopsy fall readily into the categories of natural, accident, suicide, and homicide, some deaths from natural disease appear to be influenced by stress, which raises the question of whether a manner of accident or homicide should be assigned to a death that is primarily from natural causes.

Stress as defined in these cases may include participating in an altercation, whether verbal or physical; suffering trivial injury, or nontrivial injury which is nevertheless not fatal; being afraid for one's life; fearing catastrophic outcomes to oneself or another; losing valued personal property during an assault; or undergoing poisoning with varying substances to a degree which should not be fatal, while under emotional stress from other causes.

Stress is a vaguely defined word which has been used in the media, in lay discussions, in psychology, in research, and in forensics. It has multiple overlapping meanings more than one of which may be utilized in a discussion, resulting in decreased communication clarity. Nevertheless the death of an individual during an emotionally violent incident from what appears to be natural disease, without sufficient trauma to explain death, may be difficult to describe without using the word stress.

Medical examiner opinion on the role of stress in such deaths varies. Some take the position that an influence as difficult to measure

as stress should not play a role in death certification. This series of cases from a five-year period in the Tidewater district of the Commonwealth of Virginia illustrates examples of deaths which the medical examiner felt were best certified with some reference to stress. The manner of death in these cases, as well as the relevant history, toxicology, autopsy findings, and scene investigation, is reviewed with an eye to developing some common factors which belong in the evaluation of a death that is at least partly attributed to stress.

**Stress, Altercation, Death**

## G62    Investigation of Acute Oxymorphone (Opana® ER) and Ethyl Alcohol Intoxication

*Andrew W. Sexton, DO\*, 24 Regency Park Drive, Agawam, MA 01001*

After attending this presentation, the attendees would appreciate the significant impact of coordinated interdisciplinary approach in determining an acute oyxmorphone (Opana) and ethyl alcohol intoxication.

The presentation will impact the forensic science community by reporting the limitations of ELISA methodology for screening detection of oxymorphone (Opana®)

Scene investigation, circumstantial information together with thorough autopsy/toxicology and ancillary studies constitute the triad of a competent medicolegal death investigation. Herein is described the death investigation of a 28-year-old Caucasian male, whose cause of death would be classified undetermined, had the systematic aforementioned principles not been applied. The case is characterized by astute police investigative efforts, competent scene recovery and awareness of synergistic drug effects between Opana®ER (oxymorphone) and ethanol. The decedent, accompanied by friends, participated in a celebration at a local bar prior to a major social event. Following a period of marked ethanol consumption, the decedent returned to a friend's house and retired. Approximately 6.5 hours later, attempts to awake the decedent were unsuccessful; he was found to be apneic and in asystole. Resuscitative efforts were initiated and ACLS protocols followed as the decedent was transported to a nearby emergency room. The decedent was pronounced shortly after arrival.

Autopsy findings revealed a well nourished, well developed 28-year-old Caucasian male, measuring 71 inches long and weighing 160 pounds. No external or internal evidence of trauma was detected. All body organs revealed weights within normal limits, with the exception of heavy lungs indicating severe pulmonary edema. Initial postmortem toxicology indicated non-fatal concentrations of ethanol at 0.11%, 0.14%, and 0.25% respectively in blood, vitreous humor, and urine. The scene investigation did not indicate an unsafe sleeping environment. Gross and microscopic examinations were negative for gastric contents or foreign body aspiration.

The cause of death remained undetermined until a second review and reassessment of the police investigation, including witness testimonies, raised suspicion for a drug-related event. In the area where the decedent had been sleeping, a broken 40 mg tablet of Opana®ER was found in a prescription vial on a shelf. The decedent's friend stated to police he never crushed or broke the prescription tablets. Further, the friend stated that on past occasion the decedent requested his medication for experimental use. The friend denied compliance with previous requests and no inquiry was made by the decedent the night of the party.

Additional toxicological studies included directed analysis for synthetic opioids by GCMS/SIM in blood and urine. The analysis revealed oxymorphone concentrations of 95 ng/mL and 214 ng/mL in blood and urine, respectively. The UMass Forensic Toxicology Laboratory employs ELISA technology, which includes a specific assay for oxycodone, in front line presumptive screening of postmortem blood.

Oxymorphone, a metabolite of oxycodone, exhibits limited cross reactivity in this assay (at a 50 ng/mL positive cut-off concentration for oxycodone approximately two and one-half times that concentration, or 130 ng/mL oxymorphone, is needed to elicit a positive response). The ELISA result for oxycodone was therefore negative.

The cause of death was certified as acute oxymorphone and ethyl alcohol intoxication. Most notably, the drug's manufacturer cautions contemporaneous use of alcohol since oxymorphone plasma concentrations may increase as much as 270% and causes fatal overdose. Similar to OxyContin®, crushing or breaking Opana®ER tablets defeats the extended release formulation and precipitates delivery of the drug's full dose into the blood.

This case underscores the significance of a coordinated, interdisciplinary approach to competent death investigations. Absent or superficial scene investigations, cursory or incomplete autopsy examinations, and inadequate toxicological studies can undermine accurate cause of death certifications.

**Toxicology, Synthetic Opioids (Opana), ELISA**

## G63    Deaths During Police Chases

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After attending this presentation, attendees will: (1) learn characteristics of traumatic and natural lesions identified in decedents presenting for forensic evaluation after dying during police pursuit, (2) will be able to evaluate the role of toxicology, behavioral history, and time course in determining the cause and manner of death, (3) and, will be able to distinguish injuries inflicted by police from accidental or suicidal injuries incurred during the course of police pursuit and evaluate impact of such recognition on courtroom testimony.

This presentation will impact the forensic community by assisting participants in recognizing significant characteristics of trauma and other features of deaths during police pursuit by analysis of a case format presentation.

Deaths occurring during police chases require special attention at forensic autopsy. A number of issues may be raised by death during police pursuits which are relevant to the cause and manner of death. Even issues which are not directly relevant to cause and manner may influence subsequent court decisions on police actions.

The primary issue in many cases is whether police actions directly caused death. In cases of police shootings, this is obvious; in car crashes, it may be far from obvious, so much so that the question has gone as far as the United States Supreme Court. In addition, the medical examiner may be presented with a decedent who was not the person police were chasing; passengers, innocent bystanders, and pedestrians have all been killed during police pursuit.

Death during pursuit is by no means only due to motor vehicle related trauma or police shootings. A decedent whose cause of death is clearly a gunshot wound may not have sustained it at police hands. Sometimes more than one officer has shot a decedent and the question arises of which bullet is most responsible for death. In cases where gunshot wounds are not responsible, the cause and manner of death may vary widely. Blunt force trauma or sharp force trauma such as canine bites may be identified. These injuries may have been inflicted by police, by accident, or by another. Such blunt trauma may be primary in death, contributing, or irrelevant. Other accidental means of death may supervene over police-inflicted injuries when suspects flee. The time course from initiation of police chase to death is also significant and may not always be what is expected. Deaths have occurred after police chase was called off that may nonetheless be related to the history of police pursuit.

In examination of all deaths during police pursuit, careful photographic documentation is essential along with a number of other methods of documentation only some of which are routine. Collection of trace evidence may require a higher level of care than is applied in routine cases. The medical examiner's experience and judgment as well as observations are essential to separate trauma significant in death from trauma not relevant to death, and to determine the likely origin of both kinds of trauma. For example, the medical examiner is called on to identify injuries inflicted directly by police from injuries sustained by accident or at other hands. Natural disease, intoxication with drugs and alcohol, and history of behavior such as previous flight from police or suicidal ideation prior to the incident, may all be relevant; each is likely to require careful assessment during the course of forensic evaluation. Familiarity with a variety of traumatic lesions that have occurred during police pursuits ending in death assists the medical examiner with resolving questions of police responsibility and authority, and with cause and manner of death.

This series of deaths during police pursuit provides a review of accidental, suicidal, natural, and directly police-inflicted deaths occurring over a five-year period in the Tidewater district of the Commonwealth of Virginia. The causes of death include single gunshot wound, multiple gunshot wounds, motor vehicle collision-related trauma, and drowning. The documentation of injuries and disease and the process, of medical examiner reasoning which resulted in the determination of cause and manner of death are presented for each case.

**Police Chase, Death, Forensics**

## G64 Variations on a Theme: Inhalant Abuse Related Fatalities in Central New York — An 11 Year Review

*Abraham T. Philip, MD\*, Onondaga County Medical Examiner's Office, 100 Elizabeth Blackwell Street, Syracuse, NY 13210*

After attending this presentation, the attendees will be made aware of the epidemiology of the inhalant abuse related fatalities evaluated by the Onondaga County Medical Examiner's Office from 1998 to 2008.

This presentation will impact the forensic community by examining the variations in inhalant abuse related fatalities, and dispute the notion that it is usually a juvenile behavioral problem.

Inhalant abuse is the intentional or deliberate inhalation of chemical vapors, often a household product, to achieve intoxication. The commonly used chemicals are volatile solvents, aerosols, glues, paints, and lighter fluids. In inhalant abuse there is a progression from "Sniffing" - inhalation of vapors from an open container, to "Huffing" - inhalation of vapors holding a piece of cloth that has been soaked in volatile substance against the nose and mouth, to "Bagging" - inhalation from a plastic bag containing the desired substance. The prototypical inhalant abuser is a young male, between 10 and 15 years of age, indulging in inhalant abuse during school vacation times.

A study was conducted examining the inhalant related fatalities evaluated by the Onondaga County Medical Examiner's Office from 1998 to 2008, to obtain data about demographic characteristics; circumstances of the deaths; major autopsy findings; toxicology test results; and cause and manner of death (COD & MOD) formulations of these cases.

There were nine possible cases identified by the initial searches, of which two cases were deleted as not suitable for this study. Of the remaining seven cases (three female; four male) the mean age was 32.0 years and the median age was 21. There were three cases in 2002, two in 2007, and one each in 2005 and 2008. There was one case each in the months of January, February, April, June, and August and two cases in July.

Of the seven cases only one was the so-called prototypical inhalant abuser a 13-year-old male found with evidence of direct inhalation. The three female victims were aged between 18 & 21, while the remaining male victims were in the 4th and 5th decade of life. Besides the one case of direct inhalation, three cases had spray paint residue on the face, two cases had strong circumstantial evidence of inhalant abuse and in one case there was a past history of inhalant abuse. All cases below the median age had issues with scholastic performance and or depression. The cases above the median age had histories of illicit drug and alcohol abuse or psychiatric issues.

Toxicology was confirmatory in five (71%) of the seven cases. In one case the testing was limited by decomposition of the victim and in another case specimens were not submitted for an inhalant abuse test panel. The inhalant panel tests revealed 1-2 aromatic or halogenated hydrocarbons and or ketones including the following compounds with the following frequency noted in parenthesis: benzene (1), toluene (3), difluoroethane (1), and methyl ethyl ketone (2). Illicit drugs of abuse were identified in one case, lead was identified in the gasoline direct inhalation case and multiple medications (predominantly psychiatric) were identified in four (57%) of the seven cases.

In one case each the listed COD was: complications of solvent abuse; inhalation of toxic products of combustion and thermal injury; multiple drug intoxication; and laceration like incised wounds to the neck due to circular saw. In three cases the COD was: asphyxia due to (1) inhalant abuse, (2) spray paint, and (3) drowning as the cause of death. Inhalant abuse was listed in the contributory conditions of the drowning and neck trauma victims. The MOD in six cases (85.7%) was accident and one was suicide. A further review of the autopsy report determined that inhalant abuse (or variant terms) was mentioned in the summary of diagnostic finding. The cases in which the inhalant abuse was not mentioned included the victims of fire, drowning, and multiple drug intoxication.

Education and preventive efforts focused not just on teenagers, but targeted to older at risk adults, are required if inhalant abuse related fatalities are to be eliminated. Furthermore, clinical services should consider these findings to identify the at risk individuals.

**Inhalant Abuse, Huffing, Bagging**

## G65 A New Framework for Guiding Research in Forensic Entomology: Improving the Science Relevant to PMI Estimates

*M. Eric Benbow, PhD\*, University of Dayton, Department of Biology, 300 College Park, Dayton, OH 45469-2320; and Jeffery K. Tomberlin, PhD, and Rachel Mohr, MS, Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843*

After attending this presentation, attendees will increase awareness of specific basic research needs essential for refining estimates of the period of insect activity (PIA) on human remains. Furthermore, attendees will be introduced to a needed differentiation of semantics intended to improve communication among forensic entomologists, other professionals of the forensic science community, members of the judicial process, and the general public.

This presentation will impact the forensic science community by presenting a new framework for describing the aspects of entomological activity associated with human remains. After attending this presentation, the attendees will understand the need for additional research examining neglected study foci related to the PIA, specifically the interval of activity prior to physical colonization. This presentation will raise attendee awareness to specific basic research needs essential for refining estimates of the PIA on human remains.

A major component of the nature and practice of forensic entomology is assisting investigators in determining the postmortem interval (PMI). To date, the initial time of colonization that begins the defined post-colonization interval (post-CI), and includes arthropod occupation and use of the remains, has been the most relevant information for entomologically-based PMI estimates, which is concisely defined as the PIA; however, the time between death but before initial insect colonization is also a portion of the PIA and is important for cases that require accurate estimates within hours after death. For this presentation, this portion of the PIA is defined as the pre-colonization interval (pre-CI).

The pre-CI encompasses the portion of the PIA from time of death until initial physical colonization and use by insects for consumption or oviposition. Most studies that address the pre-CI have focused on nocturnal oviposition, but few have addressed other processes that influence initial insect contact and early colonizer oviposition; most notably measurable behavioral characteristics that are influenced by both biotic and abiotic factors in the environment. In addition, there is tremendous variation in the length of time and faunal succession characteristics of insect activity on a body. The interface of the pre-CI and the post-CI is defined by the time when arthropods physically colonize and begin using the human remains as a resource; as an oviposition site, habitat for finding prey or primary consumption of tissues. This interface is preceded by an acceptance phase defined by behavioral patterns of body detection and evaluation for full colonization. The acceptance phase of the pre-CI has been all but unstudied to date, but can affect estimates of the PIA. In the current state of knowledge regarding the PIA, limited scientific information can lead to interpretative differences among forensic entomologists.

The pre-CI in general, and the acceptance phase in particular, are broad areas of forensic entomology research that have been neglected, and require more rigorous and repeatable experimental design necessary to improve the entomological information relevant to total PIA, and consequently further refinement of PMI estimates. However, a common language and framework among forensic entomologists is necessary to facilitate and guide this research. To this end, a new conceptual framework is introduced to identify areas of needed forensic entomological research and propose standard terms when discussing entomological data used in investigations involving PMI estimates. This framework divides the PMI into logical components from death to body discovery including but not limited to the following: death to initial insect detection of the decomposing body (pre-CI exposure phase); the time from detection to location of the body (pre-CI detection phase); the time from body location to first oviposition (pre-CI acceptance phase); and, the time from insect colonization of the body to discovery of the remains (post-CI).

This framework identifies specific areas of research within each of these entomological phases that involve the behavioral and physical stages of insect activity on a body, and suggests which abiotic and biotic factors influence these entomological processes that can be of focused and applied studies. It is the intention of the authors to facilitate a common language and conceptual structure to improve the science of forensic entomology, an important consideration for aiding criminal investigations involving estimates of the PMI. Accordingly, this platform is used as a method for developing a common path leading from basic to applied research in the field of forensic entomology.

**Forensic Entomology, Arthropod, Insects**

## G66 The Activity of *Calliphora vicina* (Diptera: Calliphoridae) Can Alter the Morphology and Presumptive Chemistry of High Impact Bloodstains

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After attending this presentation, attendees will have a better understanding of fly artifacts, their importance when interpreting and reconstructing a crime scene, and practical applications in locating and identifying fly artifacts.

This presentation will impact the forensic community by increasing knowledge of outside influences, specifically *Calliphora vicina*, on crime scenes and the means of using this knowledge to make more accurate scene reconstructions.

The study being presented examined the effect of *Calliphora vicina* on high impact bloodstain patterns and to test presumptive blood tests that could be used to differentiate between blood spatter and fly artifacts.

The experiments were conducted in microscenes (.46 $m^3$ wooden boxes) that had two walls of glass and a ceiling of plexiglass to facilitate observation and photography. Interchangeable inserts were made to allow for surface changes in the microscenes. Surfaces used in this study were combinations of indoor materials commonly found at crimes scenes. Combinations of white linoleum with white textured and painted walls (Combination 1), wood floor laminate with a wallpapered wall (Combination 2), and mid-grade carpet with light hued paneling (Combination 3) were used to demonstrate surface texture and its effect on the flies' ability to feed and deposit artifacts. High impact bloodstains were made from fresh (within 5 minutes of drawing) human blood on two walls and a pool was formed on the floor. The flies were placed in holding cages that attached to the microscene. This design provided an opportunity for the flies to choose to enter the microscene. Flies entered the microscene within 30 minutes with combinations 1 and 2. They entered the microscene within 60 minutes with combination 3. The flies remained in the microscenes for 48 hours. After they were removed, measurements, photo documentation, and presumptive chemical tests were performed. Four commonly used presumptive blood tests were used: phenolphthalein, Hemastix®, leucocrystal violet, and fluorescein.

The deposition of artifacts was evenly distributed between floor and wall surfaces within a microscene. Both male and female flies fed on the blood and deposited artifacts. Artifacts could range from completely clear, consisting mainly of water, to completely opaque, consisting mainly of blood. Regurgitation was the most common method of deposition, but defecation did occur. Regurgitated artifacts were generally small, 1-2 millimeters, with little or no tail. Defecated artifacts were of similar size to the regurgitated but generally had a tail from a few to over 20 mm in length.

There was no difference in reaction time between blood spatter and artifacts when using phenolphthalein, Hemastix®, and fluorescein. The reactions times with leucocrystal violet were generally similar although increased reaction time was seen in some instances. Artifacts that consisted of less blood fluoresced under a blue/green light when viewed through an orange filter without chemical enhancement.

**Forensic Entomology, Insect Artifacts, Blow Fly**

## G67 Rehydrating Dried Blow Fly Larvae to Reclaim Their Usefulness in Forensic Investigations

*Michelle R. Sanford, MS*, Jennifer L. Pechal, MS, and Jeffery K. Tomberlin, PhD, 2475 TAMU, Department of Entomology, Texas A&M University, College Station, TX 77843*

After attending this presentation, attendees will learn methods for rehydrating dried larval insect specimens. The impact that initial preservation coupled with drying and rehydrating of larval specimens on their length and weight as it relates to estimating period of insect activity also will be discussed.

This presentation will impact the forensic community by demonstrating how studies on methods development in forensic entomology can benefit the forensics community by being used to define protocols and standard operating procedures that can be cited and used in legal proceedings.

Ethanol is commonly recommended for preserving larval blow fly specimens in forensic investigations. Alcohol is a volatile preservative that can evaporate over time resulting in the dehydration of larval specimens or the creation of *crispy maggots* which are difficult to identify and unreliable for measurements for age estimation. In this study methods recommended for rehydrating dried museum specimens were adapted and applied to *crispy maggots* of three common North American blow fly species (*Phormia regina* (Meigen), *Cochliomyia macellaria* (Fabricus), and *Chrysomya rufifacies* (Macquart)). Length and weight of the specimens were documented throughout the process. The effect of initial preservation method was also observed by collecting replicate samples and preserving in 80% ethanol, 70% isopropyl alcohol, or with fixation by hot water killing followed by preservation in 80% ethanol. Third instar larvae were collected over the course of nine months from different animal carcasses used for teaching the Texas A&M University forensic entomology course. Individual third instar larvae from each species (n = 90/species) were measured and weighed before the preservative was allowed to evaporate. Rehydration was attempted by soaking overnight in 80% ethanol, a commercial trisodium phosphate substitute solution, or 0.5% trisodium phosphate solution after which specimens were again measured and weighed. Analysis of length and weight data with analysis of variance showed that for each species the impact of rehydration and the impact of the interaction between initial preservation and rehydration treatment significantly affected final rehydrated length and weight among the different species.

For all specimens, soaking in any of the rehydration treatment solutions restored a portion of the original larval length (mean percent difference initial–final across all species and preservatives: 80% ethanol: -10.6%; trisodium phosphate: -2.9%; trisodium phosphate substitute: 1.1%) but none of the solutions were able to restore original larval weight. The original larval length and the final rehydrated larval length were used to estimate larval age using published data sets. These estimates agreed within a few hours in many cases with individual preservation by rehydration treatment combinations more closely agreeing for some species than others. A comparison between the length-based larval age estimate and the known duration of the exposure of the animal carcasses revealed that there were large differences (percent difference between estimated and actual exposure: *P. regina*: 74% lower than actual; *C. macellaria*: 51% lower than actual; *C. rufifacies*: 150% higher than actual) which probably reflect delays in, and barriers to, colonization coupled with differences in tissue types used in published studies and this experiment.

Overall the data show that *crispy maggots* can be rehydrated and suggest that their length can be measured to obtain a length-based age estimate for period of insect activity estimates. Knowledge of the initial preservation method might also aid in selecting the most appropriate rehydration method.

Studies on methods development in forensic entomology can benefit the forensic sciences community by being used to define protocols and standard operating procedures that can be cited and used in legal proceedings.

**Diptera, Method, Length**

## G68 Patterns of Adult Blow Fly Attraction to Carrion Over Time

*Rachel M. Mohr, MS*, and Jeffery K. Tomberlin, PhD, Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475*

After attending this presentation, attendees will better understand about the length of time between the exposure of carrion to adult blow flies and the onset of fly attraction to that carrion. Attendees will also learn about the physiological age profile of adult flies attracted to carrion over time.

This presentation will impact the forensic community because it helps better quantify the length of time between exposure of a cadaver and the onset of insect colonization.

This presentation is intended to educate attendees about the length of time between the exposure of carrion to adult blow flies and the onset of fly attraction to that carrion. Attendees will also learn about the physiological age profile of adult flies attracted to carrion over time.

This information is significant for the forensic sciences community because it helps better quantify the length of time between exposure of a cadaver and the onset of insect colonization.

Forensic entomology's most common application is to calculate the length of time that a cadaver has been deceased, the total postmortem interval (PMI). Most commonly, what is actually calculated is the duration of immature insect inhabitation of the cadaver, based on the known growth rate of particular insect species - what the authors are terming the post-colonization interval (post-CI). Little attention has been paid to the time between exposure of a body to insect activity and the onset of oviposition, and in this research, termed as the pre-colonization interval (pre-CI). However, under appropriate conditions such as a very fresh cadaver, or when high temperatures lead to rapid decay, the pre-CI may represent a substantial portion of the total PMI. Adequately characterizing the behavior of the adult fly, particularly as a function of cadaver age and ambient temperature, could greatly assist entomologists in calculating the total period of all insect activity on a cadaver.

Insects arrive at a cadaver in relatively predictable succession patterns. Blow flies tend to arrive very early in the succession pattern, often within 24 hours postmortem. Adult flies found around a very fresh cadaver are usually presumed to oviposit shortly after locating it. However, since most blow flies require a protein meal in order to produce eggs, young flies may visit a cadaver long before they are capable of ovipositing. The process of producing eggs by depositing vitellin (yolk protein) into the immature ovarioles allows the physiological age of flies to be determined. By determining the ovarian status of flies, and the patterns of the groups' relative carrion usage, it can be accurately assessed how long postmortem oviposition-ready flies are found at a cadaver. The rate of ovarian development is largely dependent on fly metabolism, which in turn is significantly influenced by temperature. The higher the temperature, the faster ovaries develop, so long as the fly has obtained adequate dietary protein. Therefore, temperature is an important factor to track when estimating either the rate of ovarian development or simply the overall physiological age.

Experiments were performed evaluating the attractiveness of carrion to the common early-arriving blow flies, *Cochliomyia macellaria* (Fabricius) and *Chrysomya rufifacies* (Macquart). Pigs were killed by cranial stunning, and placed in an open field within one hour of death. At hourly intervals between dawn and dusk of the next 72 hours, ambient

temperature observations were made, and adult flies were collected from the carcasses. Flies were identified to species and sexed. All flies were weighed and placed in different weight classes. Female flies were dissected, and their ovarian developmental status determined in order to place them into five separate age groups. The post-CI and behavior pattern was evaluated for each group. The post-CI of each age group will be discussed in relationship to temperature. Complicating or retarding environmental factors will also be discussed, as well as the limitations of the findings. However, the results of this study are expected to be useful in improving the accuracy of entomologically derived postmortem intervals. Furthermore, this research shows the importance of collecting adult insects as well as immatures at a body recovery site.

**Forensic Entomology, Blow Flies, Postmortem Interval**

# G69 The Effect of Soil Compaction on Pupation Depth of *Lucilia sericata* in Soil

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After attending this presentation, attendees will understand the effect that soil compaction has on the burrowing ability of post-feeding third instar larvae of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) searching for a pupation site.

When paired with information from post-feeding larval dispersal studies, this information will impact the forensic sciences community by aiding investigators in locating entomological evidence at a body-recovery scene.

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Locating the oldest insects that develop on human remains is crucial for accurate analysis of entomological evidence. If the remains are in a late stage of decomposition, fly larvae from the first sere of succession might have left the remains and pupated in the surrounding soil. If this has happened, a PIA estimate based on fly larvae collected from the remains will not accurately represent the post-CI. Therefore, investigators must be able to locate flies that have pupated in the soil to obtain an accurate post-CI.

In this study, post-feeding third instar larvae of *L. sericata* were allowed to burrow into soil of different compactions. After adult emergence, the depth in the soil of empty puparia was recorded. Time from egg to adult emergence also was recorded. Results from this research will generate a standard operating procedure for collecting fly puparia in soil at a body recovery scene, as well as evaluate development

times of these insects in soil when using development data from previous laboratory studies.

The utility of *L. sericata* in forensic entomology has long been recognized. It is an early colonizer of decomposing remains, occurring in the first sere of succession. Due to its nearly cosmopolitan distribution, it has been widely studied in many locations worldwide, and can be considered a laboratory model for forensic entomology research. *Lucilia sericata* has been studied for its forensic implications in the context of temperature-related development, entomotoxicology, molecular identification, and now pupation behavior.

**Entomology, Pupation, Soil**

# G70 Feeding Patterns of American (*Periplaneta americana*) and German (*Blattella germanica*) Cockroaches on Pig Skin

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After attending this presentation, the attendees will understand the characteristics of *Periplaneta americana* (Linnaeus) (Blattodea: Blattidae) and *Blattella germanica* (Linnaeus) (Blattodea: Blattellidae) feeding sites on epidermal tissue.

This presentation will impact the forensic sciences community by educating attendees about cockroach feeding on human remains and variables that can affect their associated feeding behavior.

Cockroaches are voracious consumers of a wide variety of organic material and debris. They are also commonly found in and around human dwellings. Consequently, it is not uncommon to discover human remains exhibiting signs of cockroach feeding. Because cockroaches tend to feed on just the top layers of epidermis, their bites and feeding sites closely resemble second degree burns or abrasions. Postmortem injuries caused by cockroach feeding are often misinterpreted as antemortem injuries which can lead to the suspicion of foul play even when none exists.

The American and German cockroaches are two of the most common species of cockroach found in residential areas. The differences in feeding behavior between these two species of cockroach have not been characterized. Consequently, it is currently not possible to determine which species fed on a given set of remains. Furthermore, no information is available about the effects of temperature or population size on the feeding habits of either species.

Studies were conducted to observe the effects of temperature and population size on the feeding behavior of both American and German cockroaches. Pig epidermal tissue was used as a substitute for human epidermis. All cockroaches were starved for 24 h prior to the study and cockroaches not used in previous trials were obtained for each replicate. In order to understand the effects of population size on feeding behavior, 100, 150, or 200 American and German cockroaches were exposed to a 124.63 cm² area of pig epidermis for 48 h. The experiment was conducted at 27°C RH 80±10% and a photoperiod of 12:12 [L:D] h. Pig epidermal tissue exposed to each species was examined individually and not in mixed cultures. The effects of temperature on feeding behavior were tested using groups of 150 American or German cockroaches. One hundred and fifty individuals of each species were kept in growth chambers maintained at 15°C, 21°C, or 27°C. All growth chambers had RH 80±10% and a photoperiod of 12:12 [L:D] h. Pictures of the pig skin from both studies were taken with a digital camera every 6 h for 48 h. Feeding sites were identified and measured using SigmaScan Pro 5, and percent area damaged due to cockroach feeding was determined.

According to these studies, the amount of epidermis damaged due to cockroach feeding was positively correlated to both density and temperature. Epidermis that was exposed to 200 of either species of cockroach was damaged far more than skin exposed to lesser densities. Both American and German cockroaches showed very little feeding activity at 15°C, suggesting a minimum temperature for feeding. At 27°C, both species of cockroach consumed the most area. It is anticipated that the outcome of these studies will be useful in better identifying and understanding the interactions between anthropophagic roaches and humans in forensic investigations.

**Cockroach, Insect Feeding, Epidermis**

## G71 Attraction of Two Forensically Important Fly Species: *Chrysomya rufifacies* (Macquart) and *Cochliomyia macellaria* (Fabricius) to Inter- and Intraspecific Eggs

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The goal of this presentation is to elucidate the attractive mechanism of inter- and intra-specific eggs to two forensically important fly species: *Chrysomya rufifacies* (Macquart) and *Cochliomyia macellaria* (Fabricius). The evolutionary relationship between the two species will also be discussed, as well as the potential for this information to elucidate mechanisms used to initiate colonization of a resource by blow flies at the conclusion of the pre-colonization interval (pre-CI) which is described below.

This presentation will impact the forensic sciences community by: (1) explaining the importance of the pre-CI when attempting to estimate time of colonization, (2) examining the biology of, and interactions between, two forensically important fly species, *Chrysomya rufifacies* and *Cochliomyia macellaria,* and (3) investigating one possible mechanism triggering and/or inhibiting their oviposition on a resource.

Carrion represents a temporary and ever-changing habitat and food source for a wide variety of organisms. Previous studies indicate the first macrobiotic decomposers to discover ephemeral resources include blow flies (Diptera: Calliphoridae). However, their arrival does not necessarily translate into immediate colonization of the remains. Therefore, the period of insect activity (PIA) is broken into two portions. The pre-CI is from the time of death until arrival of arthropods on the corpse. The post-colonization interval is from colonization of the remains until discovery. Colonization can be defined as utilizing a resource as a habitat or for offspring development.

Blow flies arrive in predictable patterns and are present for a predictable time interval depending on abiotic and biotic factors. These primary colonizers may colonize carrion within hours of death. The act of colonization starts a "biological clock" and given the collective knowledge of blow fly biology, it is possible to determine the post-CI of the PIA. Since the majority of blow fly species do not colonize living tissue, exposure interval of the remains may be synonymous with the post-CI or minimum postmortem interval (PMI). In some instances estimates of the post-CI are analogous to the PMI.

The accuracy of estimating the pre-CI along with the full PIA may provide greater understanding of the period of exposure of the remains and more accurate estimates of the PMI. While a great deal is known about a few species of forensically important arthropods, much more research is needed. The community of necrophagous insects differs between habitats and between geographical areas. These differences mean that general successional and life history studies may be of some use to all forensic entomologists, but accurate PIA is dependent upon intimate knowledge of the community makeup, specific successional patterns, and life histories of forensically important flies common in the area of the crime.

Ten forensically important species have been collected in Brazos County, Texas, USA and deserve further investigation: *Calliphora livida, C. vicina, Cynomyopsis cadaverina, Lucilia cuprina, L. eximia, L. coeruleiviridis, Cochliomyia macellaria, Chrysomya rufifacies, C. megacephala,* and *Phormia regina.* Of these, *Cochliomyia macellaria* and *Chrysomya rufifacies* dominate the maggot mass during the warmer months, and are therefore important in time of colonization estimates.

Recent studies have characterized an ovipositional phenomenon in a family closely related to blow flies. Female *Musca domestica* (Linnaeus) (Diptera: Muscidae) utilize bacterial volatiles present on conspecific eggs to mediate oviposition preference. Due to the ubiquity of bacterial symbionts in the insect realm and the related life histories of Muscidae and Calliphoridae, this same oviposition mechanism may be present in forensically important blow flies, and may therefore be important to the post-CI. The current study was designed to test volatiles emitted from Calliphoridae eggs.

In this study, the attractiveness of inter- and intraspecific eggs to adult flies was investigated. A y-tube was used to present individual males and females of each species with a choice between egg clusters of each species and blank controls, and the preference recorded. The results help elucidate the mechanism for oviposition choice and timing, and help characterize the pre-colonization interval.

**Entomology, Diptera, Calliphoridae**

## G72 Effects of Resource Age and Sterilization on the Attraction of *Cochliomyia macellaria* (Fabricius) and *Chrysomya rufifacies* (Macquart)

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After attending this presentation, attendees will understand the attraction of two forensically important blow flies, *Cochliomyia macellaria* (Fabricius) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), to different resources. This presentation will also serve to educate attendees about the roles bacteria on dead tissue serve to attract flies.

This presentation will impact the forensic community by demonstrating the need to exercise caution in estimating the period of insect activity (PIA) because of the different rates of colonization by different species. By understanding differences in colonization between species, more accurate period of insect activity (PIA) estimates may be developed.

*C. macellaria* and *C. rufifacies* are two species of forensically important blow flies whose interaction is important to understanding both their behavior and their impact as evidence in forensic investigations. When both species are present on decomposing animals or bodies, *C. rufifacies* larvae commonly prey on *C. macellaria* larvae. Furthermore, studies on carrion succession suggest that *C. macellaria* is a primary colonizer of carrion, while *C. rufifacies* is a secondary colonizer. However, these studies have all provided only anecdotal conclusions about their colonization behavior.

Blow flies locate carrion primarily through odor signals given off by the decomposing tissue which combine with visual cues to attract flies to a resource. Female flies use these signals to locate the most suitable oviposition location. The aim of this study was to examine the preference of *C. macellaria* and *C. rufifacies* for resources at different

ages of decomposition; to determine if fly preference is due to the microbial content of the resource; and to determine if bacteria isolated from the resource is attractive to blow flies.

A Y-tube olfactometer was used in these experiments. In the first experiment, males and females of both species were allowed to choose between fresh and 3-day old beef liver. The second experiment consisted of testing the preference between sterile and non-sterile beef liver of different ages. In the final experiment, both species were given the choice between sterile nutrient agar and agar colonized with bacteria from the liver. Initial choice, choice after 5 minutes, and residence time at each resource was recorded in each experiment.

Chi-square analyses of the data indicate no significant preference in either species to a resource based initial response. Therefore, final response (i.e. after 5 minutes) was tabulated. *C. macellaria* and *C. rufifacies* exhibited significant preference for fresh liver and 3-day old liver, respectively. Closer analyses of the data indicate males of both species demonstrated a significant preference, but females did not. For experiment two, neither initial nor final choice showed a significant difference between species. Finally, neither species exhibited a significant preference between sterile agar and agar treated with bacteria.

T-test analysis of residence time provided similar results to those determined with the Chi-square tests. Both species spent a significant amount of time at a particular resource. *C. macellaria* resided a significant amount of time on fresh liver while *C. rufifacies* had a greater residence time on 3-day old liver. Additionally, both species spent a significantly greater amount of time on the bacteria and agar than on the sterile agar.

The results from the first experiment support the assumptions that *C. rufifacies* is a secondary colonizer and *C. macellaria* is a primary colonizer. While only anecdotal information was previously available, the data collected in these studies provide a better understanding of the behavior of these blow flies. In some cases, succession data are used to estimate the PIA. For instance, these data are useful in the case of predicting sequence of colonization by *C. macellaria* and *C. rufifacies*. Data from the studies indicate that odors from decomposing remains as well as associated bacteria might be regulating attraction and colonization by these species.

**Forensic Entomology, Blow Flies, Period of Insect Activity**

## G73 Factors Affecting the Rate of Decomposition of Pig (*Sus scrofa*) Carcasses During a Period of Drought in Southern-Western Australia

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After attending this presentation, attendees will have an understanding of the factors which affect the rate of decomposition of pigs in southwestern Australia. These decompositional data can be used to better understand the factors which affect decomposition of a human body.

This presentation will impact the forensic sciences community by illustrating the factors which will affect the time since death estimations used by law enforcement agencies. These factors will significantly affect a body exposed to environmental variables for any amount of time in Western Australia. The experimental methodology can be utilized in other parts of the world to determine the impact of local environmental factors on the rate of decomposition.

Estimation of time since death is an important factor in forensic investigations and the state of decomposition of a body is a prime basis for such estimations. Environmental factors have been shown to have a significant influence on the rates of decomposition; these factors can include temperature, solar radiation, rainfall, humidity, physical placement, coverings, and scavenging activity. Many studies have documented and quantified the influence of such factors on the decomposition stages of human bodies and pig carcasses which serve as models of human bodies in North America. However the application of these types of investigations to an Australian environment is still rare. This study provides a quantitative analysis of the impact of environmental factors on the rate of decomposition of exposed pig carcasses in the southern region of Western Australia surrounding the capital city, Perth. Pig (*Sus scrofa*) carcasses of approximately 45 kg were placed in four different environments including native bushland and suburban agricultural land. The carcasses were not protected and had trauma from the headbolt or rifle shot to the skull. The decompositional process was monitored using time-lapse image capture from an infrared camera. Monitoring was conducted for 24 hr cycles until the carcasses reached the skeletonization stage of decomposition. The images were viewed to determine the stage of decomposition and to identify any animal necrophagic activity. Weather data were collected for each location which included temperature and rainfall. This research found that temperature was the most influential factor in determining rates of decomposition with summer having significantly faster rates than any other season. While winter had the slowest rates of decomposition it was also the season with significantly higher levels of rainfall. Scavenging by native and introduced animals significantly affected the rate of decomposition in the cooler months of the year but had no significant impact in the warmer months. The lack of rainfall in all seasons except winter made statistical analysis inconclusive as to the significance of rain on the rate of decomposition. During these experiments, southwestern Australia was experiencing one of the greatest periods of drought in recorded history. Therefore the research examines both the decompositional rates in Western Australia and these rates in periods of drought.

This presentation will include methodology which can be used in other locations throughout the world to replicate the experimentation as well as the results of the study illustrating the importance of such research.

**Decomposition, Taphonomy, Western Australia**

## G74 Associative Learning of *Cochliomyia macellaria* in Response to Larval Resource: Inter- and Intraspecific Resource Interaction, and Presence of Inter- and Intraspecific Larvae on a Resource

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After attending this presentation, attendees will understand the concept of associative learning as it pertains to the blow fly, *Cochliomyia macellaria*. Experiments assessing adult blow fly response to its larval food resource, exposure of both intra- and interspecies to a food resource, and the presence of both intra- and interspecies larvae on a food resource were examined.

This presentation will impact the forensic science community by being the first to assess the importance of blow fly biology as it pertains to the pre-colonization interval (pre-CI) in a forensic investigation.

The pre-CI is the portion of the period of insect activity (PIA) prior to colonization of a food resource. The location phase begins when the insect detects a body and is more than likely governed by volatile odors

not only from the corpse itself, but also from other adult blow flies and their larvae present on the corpse. The acceptance phase begins when the insect first makes physical contact with the food resource. Understanding blow fly behavior under various conditions might allow for more concise estimates of the pre-CI of a body; the current study assesses *C. macellaria*'s behavioral response to three such conditions.

The first experiment addressed whether *C. macellaria* adult flies will be more attracted to food resources on which they were raised. An abundance of *C. macellaria* eggs was gathered from pre-existing colonies and randomly distributed between bull testicles and beef liver and kept under the same conditions. Once the flies reached the adult stage, they were only provided with water and a powdered milk and sugar mixture *ad libitum*. Beginning on the seventh day post-emergence, seven testicle-fed males and seven testicle-fed females were placed individually in a Teflon dual-choice olfactometer and their response to the resources provided recorded; likewise, seven liver-fed males and seven liver-fed females were examined under the same circumstances. Testicles were placed in containers connected to the dual-choice olfactometer, while liver was placed in the other. Resources were rotated between arms with each replicate. The olfactometer was also cleaned between sessions. This regime lasted for five consecutive days. The goal was to determine whether or not adult flies would associate with the odors of the source on which they were raised, thus "choosing" that particular resource.

The second experiment determined whether *C. macellaria* flies are equally attracted to a food resource that has been exposed to adults of the predatory species, *Chrysomya rufifacies*. An equal number of male and female *C. macellaria* were kept in one cage, while an equal number of male and female *C. rufifacies* were kept in a second cage, under the same conditions. Containers of beef liver were introduced to each cage, and one from each of the cages was removed every 24 hours for five consecutive days. Once one 24-hour exposed container was removed from each cage, they were connected to separate arms of the dual-choice olfactometer. Five female and male *C. macellaria* adults were tested to see whether they were deterred from the liver that had been exposed to *C. rufifacies*. Other containers of liver were exposed to each of the fly species colonies for 72-hour time intervals, at which point they were also used in the olfactometer. All *C. macellaria* adults used in this experiment were reared on beef liver.

The third experiment assessed whether the presence of intra- and interspecies larvae affected *C. macellaria*'s attractiveness to a food resource. This study is applicable to forensics because it addresses whether or not *C. macellaria* adults are less likely to lay their eggs on a cadaver that has already been infested with the predatory species, *C. rufifacies.* A similar experimental design with the olfactometer as described previously was used in this study. One container of beef liver containing third instar *C. macellaria* maggots and one container of beef liver colonized by *C. rufifacies* maggots were placed at the arms of the olfactometer. Five *C. macellaria* adults from each sex were tested for five consecutive days.

These experiments are the first to assess the importance of blow fly biology as it pertains to the pre-CI in a forensic investigation. In other words, the current experiments take into consideration a variety factors which may influence the colonization of a food resource by the blow fly, *C. macellaria.*

**Forensic Entomology, Period of Insect Activity, Associative Learning**

# G75    Attraction and Repellance of Blow Flies to Intra- and Interspecific Fecal Bacteria

*Melinda K. Dooley\*, and Jeffery K. Tomberlin, PhD, Department of Entomology, TAMU 2475, College Station, TX 77843-2475; and Adrienne L. Brundage, MS, 2001 Cobblestone Lane, Bryan, TX 77807*

After attending this presentation, attendees will have a greater understanding of the conspecific and interspecific interactions of blow flies: specifically, the role of fly feces and related bacteria on the attraction and repellence of forensically significant blow flies (Diptera: Calliphoridae).

This presentation will impact the forensic community by improving understanding of factors that can affect attraction and oviposition of two of the most common blow flies in the summer in the southern United States, *Cochliomyia macellaria* (Fabricius) and *Chrysomya rufifacies* (Macquart).

The most important duty of the forensic entomologist in a death investigation is to provide an estimate of the time of colonization, or period of insect activity (PIA) which translates into a minimum postmortem interval (mPMI). Blow flies are the most significant insects in death investigations because known patterns of larval development allow entomologists to determine how long a corpse has been colonized. As blow flies generally only oviposit on a body after death occurs, the amount of time that has passed since eggs were laid is the minimum length of time for which the victim has been dead. However, flies may not oviposit immediately at the instant of death. Rather, there are elements that delay oviposition, especially in *C. rufifacies*, which has been observed to arrive at the scene of death first, but only oviposit after other species such as *C. macellaria*. These elements may include quality or decomposition of the resource, the presence or lack of certain bacteria, or conspecific and interspecific signals left in the secretions of flies.

In this study, fly specks were gathered from recently emerged *C. macellaria* adults. A saline solution of the fecal matter was grown on nutrient agar, and the resulting bacteria were cultured and used in preference testing via Y-tube olfactometry. Based on odor alone, fecal bacteria do not produce volatiles strong enough to attract or repel adult flies of either species. However, certain signals must be present to trigger the beginning of oviposition of these two species on their respective timetables.

*C. macellaria* are typically one of the first species to colonize a resource in the southern United States. However, a few days after death, the majority of maggots on a body may be *C. rufifacies*. Although this species is often one of the first to arrive at a scene of death, adult females will wait to oviposit until after other fly species have begun to colonize the resource. It is possible that the presence of maggots of primary colonizers such as *C. macellaria* somehow prepares or alters the resource, improving the viability of the later-colonizing *C. rufifacies.* The adult *C. rufifacies* may be waiting for some signal that the resource has been colonized by other species before beginning oviposition, and this study investigates the role that *C. macellaria* feces and related bacteria may play in that signaling process.

By increasing understanding of what delays and triggers oviposition on a body, improved estimates of pre-colonization intervals will lead to more accurate estimates of the PMI.

**Blow Fly, Postmortem Interval, Oviposition**

## G76 Three Dimensional Polygonal Model Visualization of *Lucila sericata* From SEM and Stereomicroscopic Data

*Jeffrey R. Christiansen, BS*, Halff Associates, 1201 North Bowser Road, Richardson, TX 75081; and Jeffery K. Tomberlin, PhD, Department of Entomology, TAMU 2475, College Station, TX 77843-2475*

After attending this presentation, participants will be able to understand how a true color, three dimensional polygonal model visualization of *Lucila sericata* can be produced from SEM and stereoscopic photomicrograph data.

This presentation will impact the forensic sciences community by providing a tool for better anatomical training methods of forensically important insect species.

Blow flies (Diptera: Calliphoridae) are a cosmopolitan group of insects and often the first to colonize human remains. Therefore, they are often collected as evidence. Analysis and prediction of their age often is interpreted as the period of insect activity (PIA). However, in order to utilize any insect collected from human remains as evidence, they must first be identified. Skills necessary for identifying these insects are gained primarily through courses taken while in college, graduate school, or workshops. Primary information utilized for identifying these insects is found in texts or research publications. These sources contain detailed taxonomic information about each of these species which enables their identification. However, few resources are available that provide three-dimensional imagery for teaching or identification purposes. For this reason, a highlydetailed three-dimensional polygonal model of the species has been created. The creation of an anatomical training tool that can be utilized by any age group would significantly increase the awareness of discipline-specific species. While utilizing two different diagnostic microscopes (i.e. scanning electron microscope (SEM) and dissecting microscope), a better understanding of the anatomical characteristics and landmarks can be understood. The issue becomes, who else can benefit from the data acquired? Usually the investigator is the only individual to benefit. From this stereoscopic data, an accurate three dimensional polygonal model was created using computer software forming the basis of the three dimensional investigational/visualization tool. By combinatorial investigation, a tool can now be utilized by everyone in the form of three-dimensions, true color, high-definition imagery and movies. This proposed model is the first investigational process utilizing both stereoscopic photomicrographs and SEM data to generate a specie-specific three-dimensional polygonal model. *Lucila sericata* is a green bottle fly that is common throughout the United States during the warmer months of the year and has been used in many studies to understand the biology and ecology of blow flies in general. Therefore, this species was selected for the study.

This study is also important because it allows forensic entomologists to better communicate blow fly anatomy to a wide array of sciences including but not limited to pathology. The end result, a three dimensional visualization of the blow fly, offers a compelling tool for teachers at all levels to introduce entomology in the classroom. This concept will continued to be investigated for a further detailed polygonal model, and to include other forensically significant species.

**Lucila sericata, Visualization, SEM**

## G77 An Unusual Case of Homicidal Chest Trauma Using a Golf Club as a Weapon

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The goal of this presentation is to describe and discuss an unusual case of homicidal chest trauma caused by a single blow to the chest with a golf club that was used as a weapon.

This presentation will impact the forensic sciences community by demonstrating an unusual mechanism of chest trauma and death produced by a golf club head without penetration of the thoracic cavity by the weapon.

Different accidental injuries from golf equipment have been reported for adults and children. The vast majority of these reported cases were accidental blows to the head from clubs and balls. To this date there are no reported cases in the literature of homicidal chest trauma using a golf club as a weapon. Golf clubs are potentially lethal weapons when used inappropriately. A golf club is particularly designed to hit a golf ball. The club head is capable to accelerate to a great speed. This speed is produced by body motion swinging the club in vertical, circular, and horizontal directions.

This witnessed case involved an 18-year-old, black Hispanic healthy man who received a single blow to the chest in the presternal region with a club head during a fight. Immediately after he was hit he collapsed at the scene. Minutes later he was pronounced dead on arrival at the emergency room.

At autopsy the body corresponded to a well-developed and well-nourished lean male. He was 67 inches tall and weighed 118 pounds. External examination of the anterior torso, disclosed the presence of two well-defined brown-tan abrasions in the medial aspect of the left pectoral region separated by a 1" by 1" inch contused area. One of the abrasions was lateral and higher compared to the other. It measured 3/4" by 1/2" and had a rectangular shape. The other abrasion measured 5/8" by 5/8" and had a triangular configuration. The contused area had a triangular shape with a vertex pointing to the medial aspect of the thorax. The body had no other external signs of trauma. Upon reflection of the skin of the anterior thorax, a localized 1 ¼" by 1" hemorrhagic area was involving the presternal soft tissue and was associated to linear non-displaced fractures of the anterior aspects of the left $5^{th}$ and $6^{th}$ ribs at the costo-sternal junction. The right pleural and pericardial spaces had 1000 ml and 30 ml of liquid blood respectively. The pericardium had an extensive laceration associated with two parallel transmural lacerations of the anterior right ventricular wall, slightly parallel to the heart axis. There was no other cardiac involvement by trauma. The rest of the thoracic and abdominal organs had no lesions. Additional autopsy findings were remarkable for right lung collapse and brain edema. Toxicological evaluation was negative for alcohol, cocaine, opioids, and canabinoids.

Chest trauma is traditionally described as blunt or penetrating. The trauma is classed as blunt when the chest wall remains intact and as penetrating when the integrity of the chest wall is breached. Blunt trauma is more common than penetrating chest injury, accounting for more than 90% of thoracic injuries. Two mechanisms occur in blunt trauma: by direct transfer of energy to the chest wall and thoracic organs and by differential deceleration, experienced by thoracic organs at the time of the impact. A direct blow to the thoracic wall produces crush and shear injury associated with fractures of bones and soft tissue damage. Ribs may be fractured at the point of impact and damage the underlying

thoracic organs by producing contusions or punctures. This case represent blunt chest trauma in which a great amount of energy was applied over a small body surface causing a penetrating injury of the heart by fractured ribs. An important feature of this injury is that the fractured ribs were not found displaced at autopsy examination. A temporal displacement of these ribs could explain the nature of the heart injury.

Factors such as golf club design and physics of chest trauma are keys for understanding the mechanisms of trauma involved in this unusual homicide case.

**Golf Club, Chest Trauma, Homicide**

## G78 Non-Chemical Suffocation Deaths in Forensic Setting: A Six Year Retrospective Study of Environmental Suffocation, Smothering, Choking, Traumatic, and Positional Asphyxia

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After attending this presentation, attendees will be afforded a six-year review of forensic autopsies of non-chemical suffocation deaths in the province of Quebec, Canada.

This presentation will impact the forensic community by providing evidence-based data to support common knowledge on non-chemical suffocation deaths.

Suffocation has been the object of several papers but mainly case reports or cases series. Studies of subsets of suffocation deaths, limited to a specific scenery or category, have also been reported, such as suffocation in motor vehicle crashes, lethal crush/traumatic asphyxia, fatal entrapments in on-farm grain storage bins, suffocation by plastic bags, coffee thrombosis deaths, or overlaying and wedging deaths in children. However, no systematic study has ever portrayed non-chemical suffocation deaths in forensic setting. A six year retrospective study of all non-chemical suffocation cases in the laboratory in the forensic victim population aged of more than one year will be presented.

In the province of Quebec (Canada), a single centralized forensic laboratory covers the entire 7.5 million province population. Over a six year period (2000-2005) all autopsy cases performed at this laboratory were retrospectively reviewed for non-chemical suffocation deaths in the forensic victim population aged of more than one year. For each case, the type of suffocation, manner of death, gender, and age were compiled. In the case selection, cases of suffocation occurring in association with another type of trauma, such as sharp or blunt weapon, were excluded. Cases of suffocation in association with another category of asphyxia, such as hanging combined with suffocation by a plastic bag overhead were also excluded.

During the six year study period (2000-2005), a total of 96 non-chemical suffocation cases were autopsied in the forensic laboratory of the two Lab Sciences Judiciaies. This represents 2.3% of all forensic autopsies for the same period. Overall, cases were aged from two to 90-years-old (mean ± standard deviation, 46 ± 19), with similar averages for men (46 ± 19) and women (48 ± 24).

*Type of suffocation:* Traumatic /positional asphyxia ranked as the leading type of non-chemical suffocation, with over half cases (54%). Smothering and choking followed, in 30% and 14% of cases respectively. Entrapment/ environmental suffocation, on the other hand, was found in only 2% of cases.

*Gender and Age:* Overall, a strong male predominance was observed, with two-thirds of male victims. Traumatic

/positional asphyxia remained the leading type of non-chemical suffocation in male victims. However, the type distribution of non-chemical asphyxia differed in female victims, smothering being the most common type (64%), relegating mechanical/positional asphyxia to second position (32%). Choking occupied third position in both gender, with 16% and 5% in males and females respectively. As for age, the average in each type of suffocation did not seem to differ significantly.

*Manner of death:* Taken as a whole, manner of death in non-chemical suffocation is generally ruled as accidental (73%). In fact, all entrapment/ environmental suffocations and traumatic/ positional asphyxia deaths were accidental, as well as the vast majority of choking (85%). Smothering, in contrast, is associated with a higher variability of manner of death between cases: though suicide makes up the main core (17 cases), manner of death was ruled differently in 12 cases, including six homicides and five accidents. The most common form of smothering was from a plastic bag overhead (69%), with all suicidal smothering cases being related to this method.

In the last 15 years, evidence-based medicine has been advocated as a new paradigm, proclaiming that evidence from research is the best basis of clinical decisions and practice. In this global context, forensic pathology is no exception and is increasingly becoming a science and decreasingly an art. Nevertheless, there are still several areas of forensic pathology mainly based on tradition, with textbooks describing common knowledge that is not supported by modern research data. The present study is intended to contribute to evidence-based data on non-chemical suffocation deaths. Despite several case reports, case series and a few studies on suffocation subsets, this is the first time non-chemical suffocation deaths are systematically studied. Taken as a whole, the outcomes of this study corroborate the literature data, thus supporting the common knowledge with evidence-based data.

**Asphyxia, Suffocation, Manner of Death**

## G79 Death by Electrocution: Unusual Findings in a "Love Nest"

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After attending this presentation, attendees will realize the significance of a complete forensic examination of each case in order to understand the real cause of death. Sometimes the evidence found at the crime scene could lead examiners to misunderstand the cause of death, but only by collecting pathological, toxicological, immunological, and histological findings, you can be sure to correctly solve a case.

This presentation will impact the forensic science community by showing some unusual findings observed in an apartment that seemed to be a quiet love nest. Some of these findings seemed to suggest a different cause of death from the real one that was obtained through histological examinations.

A 63-year-old married man with two children was found dead in an apartment in a suburb of Bari, Italy. The apartment turned out to be a "love nest" of sorts, complete with nude pictures on the walls, erotic books, and pornographic videos and magazines. A large quantity of condoms and various types of sex toys were discovered in the drawers

throughout the premises. None of the family members of this man were aware of this apartment.

The body was found completely nude, lying prone on the floor of the kitchenette with the knees and pelvis in a flexed position. On the wall were exposed electrical wires from the thermostat of the boiler, a tool drawer on a shelf, and screwdrivers and pliers scattered on the floor around the cadaver. External inspection of the body showed no presence of any significant traumatic lesions except for some oval, slightly depressed, yellowish, grazed areas on the back of the right hand and on the external malleolus of the right ankle which resembled parchment.

An autopsy revealed a diastasis between the IV and V cervical vertebrae with light hemorrhage of the soft tissue which probably happened just before the moment of death (limine vitae) due to the anomalous position assumed by the cervical roots after the victim fell to the floor into a very confined space. It was later discovered that the victim suffered from pre-existing cervical arthrosis. Immunological investigation was carried out on the suspicion that the death may have been the result of anaphylactic shock, but the findings were negative. Toxicological investigation showed the presence of nontoxic levels of Sildenafil (the principal active ingredient in Viagra®), along with high levels of some components of a cutaneous disinfectant used in the sterilization of medical surgical instruments.

These findings, which seemed quite curious, were attributed to the possible transrectal absorption of the substance, most likely used in the disinfection of some of the autoerotic instruments discovered in the apartment. In the end, the definitive diagnosis of the cause of death was arrived at by means of histological verification carried out on fragments of skin taken from the grazed areas of the right hand and ankle which showed signs of the passage of electrical current. In particular, it was the coagulative changes of the epidermis (i.e., cytoplasmic hyperesinophilia, lengthening of the nuclei) and congestion of the small blood vessels which suggested that the subject died of electrocution.

**Electrocution, Sex Toys, Transrectal Absorption**

## G80 A Fatal Case Due to a Pitchfork Penetrating Head Injury

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After attending this presentation, attendees will have a better understanding of an unusual fatal case of penetrating cranial stub injury due to pitchfork.

This presentation will impact the forensic community due to the rarity of the deaths by pitchfork, the particular features of eye and intracranial lesions caused by the tool, and for the importance of a careful autopsy examination in order to clarify the exact mechanism of the death.

Penetrating head injuries can be the result of numerous intentional or unintentional events, including missile wounds, stab wounds, and motor vehicle or occupational accidents (nails, screwdrivers). The most common wound is a knife injury, although bizarre craniocerebral-perforating injuries have been reported that were caused by nails, metal poles, ice picks, keys, pencils, chopsticks, and power drills. Here is presented a case where a farmer was wounded with a pitchfork.

In the rural area in southern Italy, a 56-year-old Caucasian farmer was found unresponsive by his father-in-law a few meters from their farmland, with a tine of the pitchfork penetrating the right eye. He was quickly taken by helicopter to the nearest hospital in serious clinical condition and immediately accepted in the Intensive Care Unit. Glasgow Coma Scale score was three. A penetrating circular wound in the right eye was detected. The cranium CT showed a large hemorrhagic area in the right frontal-temporal-parietal lobe, hemoventricle and right to left brain shift. Subarachnoid hemorrhage and fractures of the lateral wall of

the orbital bone in the right occipital region was present. Neurosurgical treatment was performed for subarachnoid hemorrhage, but the man was pronounced dead four days after the penetrating stab trauma.

Prosecutor arranged the autopsy on the body because the circumstances of the wounding suggested that the death could have been a murder in connection with the father-in-law.

A complete autopsy was performed 24 hours after death. The external examination revealed a laceration in the external part of the upper eyelid measuring approximately 0.5 cm x 0.4 cm and surrounded by traces of reddish color, a wide subconjunctival hemorrhage and in the upper lateral quadrant of cornea a 0.5 cm in diameter circular tear. This corneal lesion penetrated in the eyeball that was removed and revealed on the lateral wall of the orbital bone a round bone defect measuring 0.5 cm in diameter that went through the orbit in the cranial cavity and exited in the anterior cranial fossa with a circular tear of dura mater measuring 0.5 cm in diameter. The brain was oedematous and was fixed for three weeks in 10% buffered formalin prior to being sectioned with coronal cuts. Dissection revealed right to left shift of the midlines structure. A circular injury measuring 0.5 cm was present in the right frontal region. This injury penetrated into the parenchyma from the base in the frontal region upwards and maintained the same diameter through the frontal end of the parietal lobes. The entire distance from the anterior cranial fossa bone defect to the parietal lobe measured 7 cm.

Wide foci of hemorrhages were present in the right hemisphere and characteristic petechial hemorrhages continuing throughout coronal cuts. Examination of the other organs was unremarkable. Routine histological investigation applying haematoxylin and eosin staining was performed on various organs and revealed a detachment of the upper epidermal areas mainly extends through the basal-cell layers with flattened and stretched epidermis on the eyelid skin. The deeper parts of stratum papillare and underlying upper layers of the corium were characterized for wide erythrocytes accumulation. The eye samples collected on the round laceration were stained with trichromic dye and presented the discontinuation of corneoscleral coat, choroids, until posterior camera and vitreous space with wide spread erythrocytes infiltration. Brain sections showed intraparenchymal diffuse hemorrhages.

The examination of the pitchfork showed a perfect compatibility with eye and intracranial lesions. No fingerprints from the father-in-law were collected on the pitchfork.

According to the autopsy findings and histological data, death was attributed to brain hemorrhages. The tool that caused the death was the pitchfork, and the mechanisms of trauma were consistent with an accidental trauma.

Furthermore, the circumstantial data confirmed the hypothesized death scene: it was an accidentally self-inflicted stab penetrating injury due to pitchfork.

**Pitchfork, Self-inflicted Stab Lesions, Penetrating Head Injuries**

## G81 Head Injury Associated With Posterior Distraction of the Spine in a 4.5 Months Old Baby: Analysis of the Lesional Mechanisms

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After attending this presentation, attendees will understand the analysis of lesional mechanisms in association with lesions of head and spine in a young baby.

This case report concerns a 4.5-month-old boy measuring 62.5 cm and weighing 5.5 kg who was treated by an emergency team for a cardio-respiratory arrest at the parent's house. He was declared dead upon arrival at the hospital. The first explanation by the mother was that the child had been dropped and it suffered an accidental fall down the stairs. Quickly, the theory of an accident fall was denied by the two parents.

The postmortem CT scan of the entire body showed a right parietooccipital fracture with cerebral lesions. The medicolegal autopsy found a contusion with abrasion of parietooccipital region with a bending of head, a huge hematoma associated with bone defect, diastatic fracture and extradural and subdural hemorrhage, cerebral contusions and oedema. Skin ecchymosis (three ecchymosis in right temporal region and one ecchymosis in left frontal region) was also noted. The dissection of the spine by posterior incision showed a fracture of the right part of the neural arch of T12 with extra and subdural hemorrhage of the medullar cord associated with haematic infiltration of the posterior part of the intersomatic spaces extensive on 7 cm and a small ecchymosis of the anterior part of T12 body. Because of the initial story indicating the child fell, an opening of the joints of the four limbs was performed and no macrocospical lesion were noted. The knee and wrist joints were removed for anatomopathological analysis. There was no congestion of the internal organs.

The anatomopathological findings confirm the macroscopical description consistent with premortem lesions. They pointed out an infra-clinical fracture of the right knee and a haematic infiltration of the left radio-ulnar membranous.

The spine lesions were consistent with impact of the parieto-occipital region associated with a violent anterior flexion of the spine leading to posterior distraction lesion of the spine. The infra-clinic lesion of the right knee and the left wrist suggest a violent projection of the baby against a hard surface (like a wall) followed by a fall.

This case report shows the significance of carrying out a complete dissection of the spine and the spinal cord and performing an opening of the limb joints and a removal for anatomopathological analysis in cases of suspected of non accidental injury in a baby.

**Child Abuse, Lesional Mechanisms, Head and Spine Injury**

## G82 Genetic Testing of Sudden Cardiac Death Victims: From a Forensic to a Multidisciplinary Approach

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After attending this presentation, attendees will learn a practical, ethically, and legally acceptable approach to cases of sudden cardiac death thought to be of genetic etiology.

This presentation will impact the forensic community by demonstrating an interdisciplinary approach in cases of sudden cardiac death believed to be related to channelopathies.

Sudden cardiac death is considered as the most important cause of death in western countries. In cases of sudden, unexpected deaths and especially in young people, a forensic autopsy is required, frequently followed by complementary investigations, in order to determine the cause of death. However, it happens that even after an autopsy is performed in accordance with international recommendations, the cause of death remains unexplained. Such cases, called also autopsy negative sudden deaths, are not rare (6% to 40%) and are often considered to be due to a sudden cardiac arrhythmia.

Thanks to the progress made in molecular biology, it is admitted that most cases of sudden cardiac death of children and young adults are related to genetically determined cardiac diseases. Some of them have a morphological substrate at autopsy as hypertrophic cardiomyopathy. But those related to channelopathies are impossible to detect without genetic analysis. Postmortem genetic testing referred to as molecular autopsy was recently carried out by many authors in cases without morphological explanation of the sudden death and allowed to identify pathogenic mutations described already in clinically known arrhythmic syndromes. However, it is also possible to perform genetic testing to refine the diagnosis of a hypertrophic cardiomyopathy in cases without evident morphological substrate. The genetic cardiac disease may explain the death, but it may also be at the origin of a traffic accident with a loss of car control or drowning. The channelopathies may also be involved in cases supposed to be related to intoxications. Therefore, it is important to consider the genetic screening in forensic investigations.

The legal and ethical aspects of genetic testing in forensic investigation are complex. In Switzerland, the investigating magistrate may mandate genetic testing in the forensic context in order to determine the cause of death. In fact, the particularity of medicolegal autopsy is that during the investigation procedure and in contrast to a clinical context the genetic tests can be carried out without the consent of the dead person or proxy consent. The consent is however necessary for any research activity. Genetic screening is important to establish the cause but also to detect the asymptomatic carriers in order to prevent sudden death in other family members. This prevention involves a multidisciplinary collaboration. In Lausanne, such collaboration was established between services of cardiology, medical genetic, toxicology, and forensic medicine.

This presentation will be illustrated by autopsy cases for which the interpretation of the results of the genetic screening is explained in the light of other autopsy findings. The interdisciplinary collaboration as well as the juridical and ethical aspects of genetic analyses in cases of sudden cardiac death will also be briefly discussed.

**Sudden Cardiac Death, Channelopathies, Molecular Autopsy**

## G83 Unexpected Death of 24-Year-Old Male With a Phenotype Strongly Suggestive of Lujan-Fryns Syndrome

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After attending this presentation, attendees will be acquainted with features of a rare genetic disorder which may be of great interest in common forensic practice, but at the same time is very difficult to be recognized.

This presentation will impact the forensic community demonstrating that in certain cases sudden and unexpected death could be attributed to peculiar genetic disorder which has not been described until now in forensic literature.

The presented case concerns unexpected death of a 24-year-old male shortly after his discharge from the hospital where he was admitted due to severe psychiatric behavioral disorder. He had been treated in the course of three months with the following final diagnoses: moderate mental retardation, non-organic non-specified psychosis, and significant behavioral disorder which needs special care and treatment. He was sent home in the phase of psychiatric symptoms' remission. Heteroanamnestic data obtained from the family members revealed his

cognitive and behavioral impairment many years before hospitalization; in addition, they described the physical status of the young man two days after his hospital discharge-frequent vomiting, severe abdominal pain, fever and exhaustion. Thus, he refused to eat and *ante finem* he even couldn't open his mouth. Together with other family members, his mother tried to help him by giving food and water, but all the efforts were in vain – he died in his bed. The police report confirmed all statements given by the deceased's family.

The external examination showed distinct facial dysmorphism (elongated narrow face, prominent forehead, long nose, maxillary hypoplasia, and small mandible), as well as marfanoid stature with long slender extremities. The internal examination disclosed aortic narrowing (circumference of 4.5 cm at the valves level), right aortic arch, low heart weight (240 g) with normal thickness of the left and right ventricle wall. The large intestine was widened, tensed, and full of gases and liquefied feces. No other pathological abnormalities were noticed in all other examined organs. Microscopically, slight congestion and edema of all tissues were found; specific staining for elastic fibers (Verhoeff method) showed rupture of elastic lamina and scarcity of elastic fibers with cystic degeneration of aortic media.

The postmortem toxicological screening detected the presence of clozapine and fluphenazine (neuroleptics) in the blood and bile, as well as clozapine and midazolam (benzodiazepine) in the stomach content. These findings fitted well the data in the medical records informing that the man received depot intramuscular injection of fluphenazine decanoate (25 mg) three days before his release and was instructed to continue with his therapy at home by taking clozapine (50 mg three times a day), lorazepam (2.5 mg three times a day) and midazolam (15 mg when needed at night). In the conclusion of the autopsy protocol, the cause of death was attributed to paralytic ileus due to antipsychotic therapy applied.

On the basis of the case circumstances, collected medical records, heteroanamnestic data and the autopsy findings - including peculiar facial appearance along with both macroscopical and microscopical cardiovascular features (narrowing of aortic root and ascending aorta and mediocystic degeneration of aortic wall), there was a strong suspicion on the Lujan-Fryns syndrome. In the available forensic literature the case of the Lujan-Fryns syndrome with fatal outcome is not found.

The Lujan-Fryns syndrome is defined by X chromosome-linked mild to moderate mental retardation, distinct facial dysmorphism (long narrow face, prominent forehead, long nose, maxillary hypoplasia, and small mandible), marfanoid stature with long slender extremities and behavioral problems. The genetic defect is not known; therefore the diagnosis is based on the presence of the clinical manifestations.

**Lujan-Fryns Syndrome, Paralytic Ileus, Antipsychotics**

# G84    Fatal Air Embolism During Hemodialysis

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By attending this presentation, attendees will learn of this extremely rare complication of dialysis, understand the most likely cause, and the difficulties in verifying the diagnosis.

This presentation will impact the forensic science community by reminding them of this rare complication (almost exclusively related to human frailty thwarting an otherwise foolproof system), its presentation and diagnosis.

Systemic air embolization during renal dialysis is so rare it is not referenced in any major neurology text since 1976. The most recent articles found by computer search even mentioning this complication are from 1985 and 1989.[1, 2] This reflects the effectiveness of safety devices built into hemodialysis systems in recent decades. Air embolism can occur, however, in dialysis setting through improper use or technique with venous access outside the monitored system.

A 53-year-old man had undergone renal dialysis thrice weekly for three years because of end-stage renal disease. While on dialysis his general health otherwise was good. He was normotensive, without evidence of cardiac disease, and with normal blood glucose determinations. He had been evaluated and approved as a candidate for renal transplantation and was awaiting a donor organ.

Early on a Saturday morning he reported to the dialysis facility for his final session of the week, along with eleven other patients. This Saturday the facility was understaffed with one of two nurses (RNs) missing and one patient care technician (PCT) absent. Nevertheless, the preparation and dialysis proceeded normally for the patient through the rinse-back phase. Disconnected from the dialysis machine, he had a routine sitting blood pressure check recorded as 169/86. He then positioned himself for the routine standing blood pressure check but complained of lower extremity cramping (a common complaint in dialysis patients) and sat back down on the dialysis chair. The PCT hurriedly plugged in the line of a half-filled saline bag hanging on the machine. This is a routine treatment for post-dialysis cramps or hypertension (a BP of 86/47 was recorded).

It is important to note here that the patient is disconnected from the machine and its safeguards against air in the system. The saline bag (which should have been full) and its line are outside the machine and have no air alarm. The line from the bag is connected to an existing venous access in the forearm. The bag ran empty or nearly so and was hand pumped by the PCT to get every bit of saline out of it, while calling for help. A full saline bag was quickly obtained and replaced the empty one, but no one recalled having cleared the air from the new bag and its line.

The patient fell back in the chair, unconscious and unresponsive. He could not be revived. An ambulance was called and transported the patient to a hospital. There was no venous access during transport. On admission, he remained unconscious and unresponsive. A non-contrast CT of the head showed scattered small round low-density areas on the convexity of the cerebral hemispheres suggesting air embolism. On two subsequent daily CT studies, the air shadows were gone but massive swelling of the right cerebellum with subtentoral herniation consistent with acute infarction.

Electrocardiography suggested an anterior myocardial infarction. A cardiac catheterization showed ventricular changes consistent with infarction, but coronary arteriography showed a remarkably clean coronary arbotization, given his history, with only minimal to mild atherosclerosis and no occlusive disease through five and six bifurcations. Patent coronary arteries and a subendocardial infarct were confirmed at autopsy.

The mechanism and route of systemic air embolism via venous access will be discussed.

**References:**
1    Cohle SD, Graham MA. Sudden death in dialysis patients. J Forens Sci 1985; 30:158-166.
2    Air embolism associated with hemodialysis. Health Devices. 1989; 18(11) 406-7.

**Renal Dialysis, Fatal Complication, Air Embolism**

## G85 Traffic Accident Deaths? The Importance of Autopsy

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After attending this presentation, attendees will understand Portuguese law which determines that a forensic autopsy in victims of traffic accidents must always be performed in cases of immediate death or death without medical assistance. The importance of the autopsy in these situations is obvious, since the accident is not always the cause of death. Situations of apparent victims of traffic accidents that were suicides or homicides have been described as well as natural deaths.

This presentation will impact the forensic science community by presenting several practical cases of apparent traffic accident victims in which the autopsy presented some surprises and totally different situations from those initially expected.

The need of a full autopsy and the demanding of complementary exams in these cases, namely histological, must be emphasized, reminding the fact that several judicial errors can occur in countries where a forensic autopsy is not routinely performed. Insurance rewards can be incorrectly taken into account, homicides cannot be detected, an accidental aetiology can be given to a suicide situation.

**Traffic Accidents, Autopsy, Natural Death**

## G86 Undiagnosed Preeclampsia-Eclampsia Leading to Maternal Death

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After viewing this presentation, attendees will appreciate how common preeclampsia-eclampsia is, the necessity for early diagnosis of the disorder, and the dangers encountered when it goes undiagnosed.

This presentation will impact the forensic community by bringing attention to a frequent complication of pregnancy that can be deadly if overlooked by healthcare providers. With more attention and continuing research, this disorder will be better understood, and future mothers who suffer from the disorder will potentially be saved.

Preeclampsia-eclampsia is a common hypertensive disorder of pregnancy with significant global morbidity and mortality. This disorder can be effectively treated with early recognition, but imposes serious risks for both the mother and the fetus when left untreated. Physicians sometimes fail to realize developing preeclampsia and, as a result, place both the mother and fetus in grave danger. The cause of this disorder is currently unknown, but many different ideas have been considered. Perhaps with a better understanding of the etiology of preeclampsia-eclampsia, physicians will less frequently overlook its warning signs.

The case of an 18-year-old pregnant black female found unresponsive on a pullout sofa in her apartment is reported. The decedent had received regular prenatal care at a local hospital. Medical records disclosed that she exhibited significant proteinuria of 8.8 g/24 hr five days prior to her death, with relatively normal blood pressure measurements. Instead of being admitted to the hospital, her physician elected to send her home on bed rest. Autopsy records revealed that the decedent was 38 weeks pregnant at the time of death, with autopsy examination revealing some well-known sequelae of preeclampsia-eclampsia, including intracerebral hemorrhage and platelet and fibrin microthrombi of the kidneys that indicated a thrombotic microangiopathy. Hepatocellular necrosis was also observed. The singleton pregnancy revealed an unresponsive male fetus with no evident developmental abnormalities. The cause of death was listed as complications of preeclampsia, with extensive intracerebral hemorrhage.

**Preeclampsia-Eclampsia, Maternal, Death**

## G87 Rupture of the Left Ventricle Due to Blunt Trauma - A Pediatric Case Study

*Francois Paysant, MD*, Chu, Hopital Michallon, BP 217, 38043 Grenoble Cedex 9, Grenoble, FRANCE*

The goal of this presentation is to describe cardiac rupture following thoracic or abdominal trauma, which is relatively unknown, particularly in the pediatric traumatology.

Cardiac tamponade due to traumatic rupture of the chambers of the heart, in particular the left ventricle, after blunt thoracic trauma is described only sparsely in the literature. Most cases involve multiple thoracic trauma following motor vehicle accidents. To the best of knowledge, blunt traumatic injury following a household accident has not been described.

The case study will be presented of a five-year-old victim of a household accident, in which two concrete basins apparently fell on him. He died quickly despite attempted resuscitation.

The autopsy showed an ecchymotic scrape in the lumbar region as the only external lesion, with no bone injuries, bilateral pulmonary contusions at the base of both lungs, hemorrhagic extravasation of the diaphragm and mediastinum, hemopericardium, and massive damage to the apex of the left ventricle. Pathological exam confirmed the traumatic origin of the cardiac rupture, with no underlying pathology.

The mechanisms described in the literature that result in such lesions, the mechanism which the authors believe most probable in this case, and the importance of background information will be discussed. In this case study, lack of specific information concerning the accident prevents a definitive conclusion of the exact mechanism that caused this massive trauma particularly due to the fact that the external examination couldn't find any lesion in favor of a thoracic or abdominal traumatism. It is unknown if such an isolated case of a lesion causing almost immediate death has previously been described in the literature.

**Blunt Thoracic Trauma, Left Ventricular Rupture, Autopsy**

## G88 Postmortem Examination of Coronary Artery Stents Using a Hand-Held Rotary Tool

*Christopher Tape, MD*, and Patrick E. Lantz, MD, Department of Pathology, Wake Forest University, SOM, Medical Center Boulevard, Winston-Salem, NC 27157-1072*

The goal of this presentation is to describe a practical technique for postmortem evaluation of coronary artery stents.

This presentation will impact the forensic community by permitting evaluation of intra-luminal patency, thrombosis, and restenosis of coronary artery stents and assist in determination in assigning cause and manner of death.

Since the development of the intra-luminal coronary artery stent in the late 1980's the use of coronary stents has increased dramatically worldwide. In 2005 one or more stents were placed within coronary

arteries of 620,000 patients in the United States. Numerous clinical studies have shown the benefits of coronary artery stents in the treatment coronary artery disease; however, the main early complication with an intra-luminal coronary artery stent is thrombosis, while the primary long-term complication is in-stent restenosis. Today, stent surfaces and coatings are designed to prevent thrombogenesis and many elute drugs that inhibit neointimal proliferation to reduce in-stent restenosis. Despite advances in stent technology, stent thrombosis and in-stent restenosis remain common complications that can lead to myocardial ischemia, infarction and possible death. Discovery of clinically significant stent complications at autopsy can be crucial for the pathologist trying to determine the cause or manner of death. However, evaluation of coronary artery stents at autopsy is challenging and has been limited to postmortem angiography, serially sectioning the stent with a low-speed diamond saw or simply by visual examination of the stent lumen and estimating any luminal narrowing. Most medical examiner offices cannot afford the expense, space, or training required for postmortem angiography or a low-speed diamond saw to examine coronary artery stents.

A hand-held rotary tool can serially section coronary artery stents with minimal deformation of the stent, distortion of the luminal space or disruption of intra-luminal contents. The excised coronary artery stent is serially sectioned in 2 -3 mm increments. When laid out in cross-section from proximal to distal, the sections of the stent and surrounding coronary artery can be assessed and photographed. Luminal contents can be removed by careful dissection using 20-gauge needles. Subsequent histological evaluation can determine if the intra-luminal material is postmortem clot or premortem thrombus. Sectioning of coronary stents with a hand-held rotary tool is affordable, easy to master, and permits objective assessment of intra-luminal coronary artery stent patency, thrombosis or restenosis.

**Forensic Science, Coronary Artery Stent, Hand-Held Rotary Tool**

## G89    Detection of Wild Game DNA in Maggot Tissue

*Jill C. Ross, BS\*, and John R. Wallace, PhD, Department of Biology, Millersville University, Millersville, PA 17551; and Jane E. Huffman, PhD, Northeast Wildlife DNA Laboratory, East Stoudsburg University, East Stroudsburg, PA 18301*

After attending this presentation, attendees will learn a new technique to assist conservation or game officers in the identification of illegally harvested wild game through the detection of DNA specific to several game species in maggot tissue. The attendee will learn how to rear, collect and preserve maggots, identify insect developmental stages as well as perform molecular analyses to identify non-human DNA.

This presentation will impact the forensic community as well as game law enforcement by demonstrating that entomological evidence can be useful in criminal investigations other than determining a minimum postmortem interval. To date, molecular analyses are useful to identify game species through their DNA; however, analyzing insect tissue for the presence of animal/bird DNA may provide another technique useful in wild game management and conservation.

Poaching wildlife is a problem faced by many conservation and game officers and many people are caught and convicted each year, but it is a crime that even more offenders get away with. Annually, there are approximately 1,000 big game poaching cases prosecuted. Unfortunately, many cases do not reach the court of law due to either the lack of personnel required to patrol over 26,000 square miles of forested game lands or lack of evidence required to identify the game species in question. The use of entomological evidence in human death-scene investigations in terms of estimating the minimum postmortem interval has been well documented as well as to some degree with wildlife. In addition, insect evidence can be used to differentiate between human and animal DNA through molecular analyses of the food stuffs in maggot crops. The purpose of this study was to examine maggot tissue (crop or entire body) and determine if wild game DNA could be detected using PCR analysis. The objectives for this study were to develop protocols using current PCR technology to identify and compare wild game DNA isolated from Dipteran larvae, and determine if larval developmental stage influenced the isolation and identification of wild game DNA.

Three species of forensically important flies were reared in the laboratory (Calliphoridae: *Calliphora vicina* and *Lucilia cuprina*; Sarcophagidae: *Sarcophaga haemorrhoidalis*) on approximately 350 g of deer, bear, coyote, bobcat livers. Bear, deer, coyote, fox, and bobcat livers were obtained from either euthanized animals or vehicle strikes. They were frozen immediately after removal. Fly larvae were collected at mid-molt from each larval instar, preserved in 95% ethanol and identified for species and age confirmation. After identification, maggots were individually preserved in 1.5mL of 95% ethanol and shipped in centrifuge tubes to the Wildlife Forensics Laboratory in East Stroudsburg University for PCR analysis.

Before DNA extraction, maggots were washed to remove potential external contaminants. Each maggot was individually soaked for 2 min a 1.5mL tube containing 1mL of 20% bleach. The bleach was removed and each maggot was rinsed twice with 1 mL of sterile distilled water. Each clean maggot was with iris scissors, then a ventral incision was made from the posterior to anterior end of the maggot. If possible, the crop was removed with forceps. In some circumstances, either the entire anterior inside of the maggot was removed or the entire maggot was extracted.

Amplifications were performed using Promega PCR Master Mix.

Each reaction included 1 µL of each primer (5 pmol/µL) and 5 µL of DNA extract. The PCR program consisted of an initial denaturation cycle of 95ºC for 3 minutes, 45ºC for 1 min and 72ºC for 1 minute-30 seconds, then continued with 33 cycles of 94ºC for 1 minute, 45ºC for 1 minute and 72ºC for 1 minute-30 seconds, with a final extension at 72ºC for 3 min 30 s. The success of PCR reactions was determined using an agarose yield gel stained with ethidium bromide. Sequences were aligned and edited using Sequence Navigator software (Applied Biosystems). Quantitation of crop extractions showed the amount of DNA recovered varied with the species analyzed. The extractions produced at least 1.0 ng/µL. The samples analyzed produced the correct mtDNA haplotype for deer.

**Maggot Tissue, Wild Game, DNA**

## G90    Conversion of the Wyoming State Crime Laboratory From FM-BIO Slab Gel Technology to the AB 3130 Genetic Analyzer for CODIS and Casework Sample Analysis

*Timmy L. Neece, BS, BA\*, 1239 9th Street, Apartment 1, Huntington, WV 25701*

After attending this presentation, attendees will have gained an understanding of the work involving validation of the forensic methodology used with an Applied Biosystems 3130 Genetic Analyzer for genotyping CODIS. This validation includes the study of precision, reproducibility, concordance, sensitivity and the ability to resolved mixtures of biological samples.

This presentation will impact the forensic community due to its validation of methodology for use on an instrument and associated kit technologies is vital to obtaining precise and accurate profiles of genetic samples for case work or the CODIS database.

For a method to be validated for forensic analysis it must meet several guidelines put in place by the Scientific Working Group on DNA

Analysis Methods. The data must show a degree of precision and reproducibility based on known laboratory controls. To validate such a system it must be shown that there is no contamination between samples run on the instrument. A series of non-probative samples must be run to verify that the protocols are suitable for samples that could be encountered during forensic casework.

The validation was performed with the Profiler Plus™ and the COfiler™ allelic kits and all the samples were analyzed using the Genemapper ID™ software. To ensure the reproducibility and precision of the instrument a series of allelic ladders were injected. The allele calls and the peak heights were analyzed and confirmed that the protocols performed by the Wyoming State Crime Laboratory (WSCL) were conservative enough to meet the stringent standards necessary for forensic investigation. A series of fifteen samples were amplified, run and analyzed with the standard WSCL protocols. These samples had been analyzed before on the WSCL's AB 310 genetic analyzer and had known profiles. The experimentation with the 3130 concluded that the instrument was capable of producing profiles that matched the known profile. These studies demonsrtated that no contamination between samples injected on the instrument occurred. With each study, negative controls were performed across the board with each sample showing no contamination.

The data obtained from the validation of the Applied Biosystems 3130 at the WSCL. It is important for those using the AB 3130 genetic analyzer to be familiar with how the instrument and associated kit technologies were validated and the experimentation that was performed.
**3130, Validation, CODIS**

## G91 A Rapid Penta STR Screening Method by Microchip Capillary Electrophoresis

*Maurice J. Aboud, BSc\*, 10720 North West 66th Street, Apartment #113, Miami, FL 33178*

After attending this presentation, attendees will understand the development of a fast and portable DNA screening method that uses microchip electrophoresis for forensic DNA applications. Attendees will gain an understanding for how this system operates, the limitations of the system, and how these limitations were surmounted to achieved the desired resolution on the microchip.

This presentation will impact the forensic community by development of a multi-loci penta DNA system in combination with an STR micro-chip electrophoresis system that provides a new tool for quick and portable screening in forensic DNA analysis.

Due to the ever-increasing forensic DNA caseloads and the potential need for remote forensic DNA analysis, DNA analysis systems that are both quick and portable are needed. While short tandem repeat (STR) DNA analysis by capillary electrophoresis is capable of high resolution and has a large power of discrimination in forensic identification, these instruments are not portable and require a relatively long sample run time. It is on this basis that this study aims to develop a rapid and portable DNA screening method using a commercially available microchip electrophoretic system. Generally speaking microfluidic systems require fairly long channels and complex detection systems for proper resolution and identification of forensic DNA. However there currently exists commercially available systems, such as the Agilent 2100 Bioanalyzer, which have a small footprint and utilize chips with short channels and reduced resolution. Such portable systems might be valuable in situations where evidence screening is necessary in remote locations. However due to its lower resolution, most STRs will not properly separate on such a system. In this project the development of mini-Penta STRs as potential tools for microfluidic analysis was investigated. These five base STRs should produce more consistent results in such circumstances.

The design and development of such a portable system capable for forensic DNA screening with high enough resolution for single allele separation required that the following issues be investigated in order to increase the resolution. First the analysis of the current Penta STR markers available from Promega Corporation and redesign of these primer sets to reduce amplicon size and improve the mobility and separation within the micro-channel. Secondly, the development of a denaturing polymer for single stranded DNA separation to be used on the microchip that would take advantage of the improved resolution in single stranded DNA assays. Finally, the development of a Penta multiplex STR kit that would increase the power of discrimination for forensic samples and become a more powerful forensic tool.

These studies were designed to overcome the limitations of current microchip systems for portable forensic applications by trying to increase the resolution of the short micro-channels. It is with these changes that the resolution of the system should be capable of separating between five base pair repeats accurately and robustly.

This research will address the problems and limitations encountered with the current systems such as poor resolution, large amplified DNA fragments, and the ability to only detect double stranded DNA on the currently commercial available microchip systems such as the Agilent 2100 Bioanalyzer. As a result of this research the development of a multi loci penta DNA system in combination with an STR microchip electrophoresis system should provide a new tool for quick and portable screening in forensic DNA analysis.
**DNA, Penta STR, Microchip**

## G92 How Does Season Affect the Release of Ninhydrin - Reactive Nitrogen Into Grave Soil?

*Jessica D. Wilkerson\*, and Brianna F. Anderson, University of Nebraska-Lincoln, 202 Entomology Hall, PO Box 830816, Lincoln, NE 68583-0816; and David O. Carter, PhD, University of Nebraska, Lincoln, Department of Entomology, 202 Plant Industry Building, Lincoln, NE 68583-0816*

After attending this presentation, attendees will understand that seasonality can significantly affect the rate at which ninhydrin - reactive nitrogen (NRN) enters grave soil and that the dynamics of grave soil NRN can contribute to the estimation of postmortem interval (PMI).

This presentation will impact the forensic community by serving as a fundamental investigation into the estimation of extended PMI. Accurate estimates of extended PMI are currently difficult to achieve.

During the summer months, when temperatures are warm, bodies tend to decompose at a more rapid rate. Recent research has shown that a body releases NRN into grave soil during decomposition. At present, few studies have investigated NRN in grave soil when decomposition begins during colder months. The release of NRN has primarily been used to locate graves, but more recently, has been investigated for its use in estimating PMI. To investigate this use, researchers decomposed carcasses in winter and summer to compare the release of NRN into grave soil.

The experimental site was located at the University of Nebraska Agricultural Research Development Center located approximately 48 km north of Lincoln, Nebraska, USA. The site is a pasture that is intermittently grazed by cattle and horses. The soil at the site is a deep silty clay loam of the Yutan series (Mollic Hapludalf). The climate is temperate midcontinental characterized by hot summers, cold winters, and moderately strong surface winds. Average annual precipitation is 695 mm. Approximately 75 percent of the precipitation occurs between April and September. Mean annual temperature is 9.8°C with mean minimum and maximum temperatures ranging from 0 C (January) to 31°C (July). The vegetation at site is dominated by non-native grass

(smooth brougham) and forb (white clover) with some native vegetation, including daisy fleabane, yellowwood sorrel nut sedge, and pasture rose.

Swine (*Sus scrofa*) carcasses (~40 kg) plus a control (no cadaver) were used. Swine were killed with blunt force trauma to the cranium and placed on their right side on the soil surface facing west. Swine were killed and placed on the soil surface during February 2008 (winter) and June 2008 (summer). Soil samples were collected (0-5 cm depth) from adjacent to the cadaver at intervals of 15 days for the initial 30 days. This experiment was replicated three times, which resulted in a total of six cadavers.

The concentration of NRN during the summer months was greater than during the winter months. Elevated levels of NRN were observed during the summer months after 15 and 30 days postmortem. In contrast, a significant increase in NRN was not observed during the initial 15 days of decomposition during the winter months. These results demonstrate that NRN would not be an accurate method to test for the presence of grave soil during the initial 15 days of death. As decomposition in terrestrial ecosystems is primarily biologically mediated, this influx was likely more rapid during the summer months because of greater insect and microbial activity. A more accurate way to measure postmortem interval during the winter months would be to use degree days, which will be presented along with measurements of NRN after 60 and 90 days.

**Forensic Taphonomy, Postmortem Interval, Temperature**

## G93 Consumption of Fly Artifacts After Deposition and Translocation of Bloodstains by *Calliphora vicina* (Diptera: Calliphoridae)

*Becca Striman\*, 1347 North 37th Street, Lincoln, NE 68503; Amanda Fujikawa, BS, 202 Entomology Hall, University of Nebraska-Lincoln, Lincoln, NE 68583-0816; Larry Barksdale, MA, Lincoln Police Department, 575 South 10th Street, Lincoln, NE 68508; and David O. Carter, PhD, University of Nebraska, Lincoln, Department of Entomology, 202 Plant Industry Building, Lincoln, NE 68583-0816*

By attending this presentation, attendees will learn of two newly observed behaviors through which *Calliphora vicina* can alter bloodstain patterns.

This presentation will impact the forensic community by contributing information to the current literature regarding the behavior of *Calliphora vicina* when exposed to blood. Blow fly behavior can alter bloodstain patterns at crime scenes, which can lead to inaccurate crime scene reconstruction.

The purpose of this study was to observe the behavior of *C. vicina* when exposed to an expired bloodstain pattern and their effect on bloodstains on wallpaper, white textured wall, and white laminate floor.

The experiment was conducted using eight microscenes (0.46 m³ wooden boxes) that had two glass walls and a plexiglass ceiling to enable easy observation and documentation. The other surfaces consisted of a textured, white painted wall, a wallpapered wall, and a white laminate floor. A holding cage was attached to each microscene, in which ten flies were placed. Four of the microscenes were control scenes and no flies were placed in their holding cages. The holding cages were designed to allow the flies access to the microscene without human intervention.

Fresh human blood was used within ten minutes of being drawn. In each microscene, approximately three milliliters of blood were poured into a pool in a corner of the microscene. The donor then put three milliliters of blood in his mouth and expirated blood into the microscene. The blood was directed towards the interface between the wallpaper and white painted wall. The flies were allowed entry to the microscene for 72 hours and had access to sugar and water.

Flies moved from the holding cage into the microscene within 10 minutes and began feeding on the blood within five hours. All deposited artifacts that were observed were produced from defecation. No artifacts with long tails were made while the flies were exposed to light. Blow flies were observed feeding on fly artifacts, sometimes within seconds of the deposition of the artifact. Some of the artifacts were completely consumed by the flies. During the last half of the experiment, the flies fed on artifacts in equal or greater proportion to the bloodstain pattern. A small drop of blood was translocated by the mouthparts of the flies. The mouthparts were swept across the wall in an arc, beginning at the original source and ending at the new droplet, without leaving a trail of blood. The flies were observed feeding on the bloodstain pattern until the experiment ended.

The consumption of fly artifacts may occur because the artifacts could be easier to digest than pure blood, in the same way that regurgitated blood is easier to digest. However, defecated artifacts are unlikely to be as nutritious as pure blood. Translocated blood droplets may cause additional confusion when analyzing bloodstain patterns, especially if a reliable method is developed to distinguish fly artifacts from human blood. It is unknown how common this behavior is or whether it could significantly alter the overall bloodstain pattern. It is important for crime scene investigators to consider the behavior of blow flies when attempting to reconstruct a crime scene based on bloodstain pattern analysis. However, many more experiments are needed before this subject is thoroughly understood.

**Forensic Entomology, Expirated Blood, Blow Fly**

## G94 Decomposition of Child-Sized Remains in Dumpsters

*Kevin M. Willis, BS\*, Washington County Sheriff's Office, 1535 Colfax Street, Blair, NE 68008*

After attending this presentation, attendees will have an increased understanding of the decomposition of child-sized remains placed in a dumpster.

This presentation will benefit the forensic community, as well as those in the fields of the postmortem interval estimation and rate of decomposition research. It may benefit investigations of children killed and placed in dumpsters. The research demonstrates that a child-sized carcass placed in a black plastic bag and in a closed dumpster will decompose at a slower rate than one placed in a dumpster unbagged. Both pigs in dumpsters decompose more slowly than a control pig outside the dumpster.

The results of this research will benefit forensic science in the fields of postmortem interval estimation and rate of decomposition research. It may benefit investigations of children killed and placed in dumpsters. The research demonstrates that a child-sized carcass placed in a black plastic bag and in a closed dumpster will decompose at a slower rate than one placed in a dumpster unbagged. Both pigs in dumpsters decompose more slowly than a control pig outside the dumpster.

Research on child-sized remains has been done by depositing pigs in a variety of ways including surface deposit, shallow grave, covered by branches and debris, suspended by a rope, and rolled in carpet (Morton and Lord, 2002). However; there is not much study, if any, on the decomposition of child-sized remains in a dumpster, despite the forensic cases where children's bodies have been disposed of in this fashion. The aim of the research is to understand the environmental and taphonomic factors that affect the postmortem interval (PMI) on child-sized remains in a dumpster.

This thirty-day project began on June 15, 2008 and ended July 15, 2008. Three small pigs, which were humanely dispatched, were used as child-sized remains. Pigs were chosen because their internal structures and progression of decomposition are similar to humans. Two pigs were placed on plywood in individual dumpsters and the third was placed on plywood on the ground as a control. The control pig was not covered but protected on all sides by a chain-link fence.

A four-lead temperature coupler was placed with each pig and programmed to take hourly temperature readings for the duration of the project. Each pig was weighed daily using a digital scale and their girths measured. An incised wound was also placed behind the right shoulder of each pig.

Each board was weighed without a pig and then with a pig subtracting the difference for obtaining the weight of each pig. The two dumpsters were each two cubic yards in size with two plastic lids. The lids were kept closed except for collecting data.

The pigs in the dumpsters had temperature leads placed in the following locations: in the mouth, underneath the pig (between the pig and the plywood), hanging loose inside the dumpster and hanging outside the dumpster. The control pig had a temperature lead in the ground approximately two inches in front of the pig in lieu of the temperature lead hanging loose inside the dumpster; other temperature leads were placed in the same positions as with the pigs inside the dumpsters. Data collection was performed each day. Information recorded included weather conditions, body temperature, container temperature, and carcass weight loss. Photographs were also taken of each carcass.

General decomposition patterns were observed on each of the specimens. Fly succession following the usual pattern for the region was noted on all three specimens. As measured by weight loss, the control pig decomposed at a faster rate than either pig in a dumpster. The pig in a bag in the dumpster decomposed more slowly than the pig not in a bag in the dumpster. The initial data suggests that the decomposition rate of remains placed in a dumpster is noticeably inhibited.

**Decomposition, Dumpsters, Taphonomy**

# G95 Inadvertent Administration of Lidocaine: Illustration of Two Cases

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After attending this presentation, attendees will have viewed two cases showing that the inadvertent administration of lidocaine can lead respectively to death and to serious tetraparesis linked to loss of the cognitive functions.

This presentation will impact the forensic community by describing two cases that show how the inadvertent administration of lidocaine can lead respectively to death and to serious tetraparesis linked to loss of the cognitive functions.

**Case 1:** In the first case, a 35-year-old man who for many years has been affected by protruded disk between L4-L5 causing right lumbosciatic neuritis, during an orthopedic examination, was administered lidocaine and Thiocolchicoside injection in right paravertebral lumbar area.

Soon after the man started to feel worse, and over an hour, showed sudden loss of consciousness, seizures, acute respiratory insufficiency, arterial hypertension, and severe tachycardia. The man was transported to the local emergency room where he arrived comatose. The cerebral CAT showed small gas bubbles in the cornua of the lateral ventricles and suprasella cistern.

Despite the pharmacological treatment, the man suffered serious seizures with heart attack and subsequent death. After the family's complaint, by an order of the legal authorities, the external examination and the autopsy were performed two days later.

**External examination:** The man was 175 cm tall and his weight was 76 kg. No injuries were found in his body; the external examination showed only some puncture marks on the right wrist, on the antecubital fossae and on the back of the left hand, and subcutaneous tumefaction in right paravertebral lumbar region, on which there was a puncture mark 4 cm from the spinous apophysis of L5.

**Autopsy findings:** The forensic autopsy revealed brain edema and congestion of cerebral veins. There was no lesion in the scalp and in the galea capitis and no intracerebral hemorrhage was found. Pulmonary edema, pancreas and kidney congestion were found. The heart showed hypertrophic left ventricular and septal wall and left ventricular chamber dilatation. The section of subcutaneous tumefaction in right paravertebral lumbar region, saw in the external examination, showed a rounded formation, circumscribed by a fine membrane, of soft and elastic consistency, dark red complexion, contains a blood clot. The section of lumbar vertebrae and the following extraction of the conus medullaris allowed to find, at the level of the L5, a blood infiltration in the posterior dural sac and underlying arachnoid.

**Histological Findings:** The microscopic examination showed multivisceral congestion. Myocyte cellular hypertrophy and contraction-band necrosis of left ventricular and septal wall were observed. Severe left anterior descending coronary artery stenosis, softening of temporal cortex, white substance edema and neuronal cerebral and bulbar cytotoxic edema, spinal cervical cord edema were also noted. The terminal conus medullaris at the level of the L5 showed blood infiltration in the posterior dural sac and underlying arachnoid, soft and adipose tissue emorrhagic extravasation.

**Case 2:** In the second case, a 58-year-old woman who for ten years had cervical pain due to protruding disk between C5-C6 was submitted to lidocaine infiltration made laterally to the cervical spinous apophysis.

Soon after, the doctor noticed the progressive decrease of the radial pulse, the loss of consciousness and the cardiac activity, so he started the external cardiac massage. After 15 minutes, the emergency medical doctor made an intracardiac injection adrenaline, after which there was a restarting of the cardiac activity. The woman was transferred to a hospital where she felt into coma.

The cerebral CAT showed many gas bubbles in the suprasellar, perisellar, and temporal periencephalic space, left sylvian valley and cornua of the lateral ventricles.

Currently the woman shows a serious situation of rigid-spastic tetraparesis and loss of cognitive functions.

**Discussion:** In the first case, vascular-peridural iatrogenic inoculation and the consequent sistemical diffusion permitted the neurotoxic damage lidocaine and epileptogenic action thiocolchicoside. The toxical cerebral effects destabilized the pre-existing ischemic cardiopathia, serious but clinically asymptomatic, causing the death of the man.

In the second case, at first the anaesthetic damaged the cervical orthosympathetic chains nerve ending, leading to a reflex inhibition of spinal cardiac-vasomotor centers and consequently to a hypovolemic shock, an then produced a direct neurotoxic damage of the S.N.C., both responsible of the quadriplegia.

**Lidocaine, Coma, Tetraparesis**

## G96 Asphyxia by Confinement: The Death of a Man Kidnapped and Segregated in a Small Underground Cistern

*Cettina Sortino\*, Antonino Bonifacio, MD, Sergio Cinque, MD, Stefania Zerbo, MD, Livio Milone, PhD, and Paolo Proccianti, PhD, Department of Biotechnology and Legal Medicine, Section of Legal Medicine, Via del Vespro, n. 129, Palermo, 90127, ITALY*

The goal of this presentation is to illustrate a particular case of homicide of a 68-year-old Caucasian man who was found dead inside an underground cistern, tied with ropes and chains.

This presentation will impact the forensic community by presenting the unpredictability of death by asphyxiation from confinement of a man unlawfully restrained for the purpose of extortion.

The air of confined environments can be subject to alteration by shares of various causes. Those persons who stay indoors need to have available a sufficient volume of air appropriately refreshed.

The sensitivity of the subject varies in relation with the temperature of moving air (compared to room temperature), the direction of air current, and conditions of the subject.

In February 2007 a male corpse of the apparent age of 65-70 years was found inside a cistern built underground, three meters deep, and used to conduct the passage of water (size of 3x3x3 meters, closed tightly with a metal lid of 63x63 cm).

The analysis of clothing made it possible to identify the victim; it was a Caucasian 68-year-old man who had disappeared a month before the discovery.

From data carried out during inspection, the victim was lying on the ground and immobilized by the presence of several girdles consisting of a rope and a chain.

The rope, surrounding the sides, kept him in contact with water and blocked any possibility of his movements. The chain consisted of steel mesh fixed at both of wrists and left ankle, as follows: the right wrist was linked to the left ankle at a distance of ten mesh chain links. This position forced the bending of the left knee over 90° and the extension of the right arm, not allowing any movement of the arm or the leg.

The left wrist was also linked to the left ankle by a chain at a distance of 22 cm, allowing the bending of forearm and arm; this chain also passed below the rope tied to waist passing on the left side.

The external examination of the corpse showed chromatic-emphysematous state of putrefaction; negroid face, with disjunction of hair in large areas of the scalp, eyeballs completely concave for colliquation and evaporation, easy detaching of skin grafts; massive destruction of nasal cartilage and perioral soft tissues, with exposure of dental arches and jawbone.

There were also larvae in various stages of maturation (pupae of 1 and 2 stage) and skin erosions caused by their destructive action. Several skin areas were affected by the presence of fungal growth on the right side, in particular: the neck, the chin, the right auricle, the upper right region of chest, the stump of right shoulder, and the periumbilical region.

Some skin areas were blackish and partially wrinkled (head, neck, upper region of chest, and upper limbs), while others were affected by phenomena of maceration with detachment of skin (hands, feet, right thigh, and both legs).

There were no signs of constriction on the neck. Under the hypogastric area, umbilical region, right wrist and left wrist, there were impressions caused by the rope and the chain. The autopsy showed advanced putrefaction in all organs, in particular in the brain, pancreas, and adrenal glands.

The histological examinations made it possible to detect signs of vitality on the skin of wrists and, in particular, there was oedema and intralveolare hemorrhage swelling and bleeding intralveolare and, inside the blood wessels, the red blood cells seemed conglutinate with focal fibrin blood clots. Histological examination of the heart showed only a moderate atherosclerosis in coronary vessels.

The toxicological tests carried out on tissues and fluids have ruled out the presence of drugs and/or psychotropic substances, and showed the presence of 7% of carboxyhemoglobin.

On the basis of putrefaction, the presence larval and the conditions under which he was forced, it is estimated that the death could be traced back presumably, in a variable range of about 20 days before its discovery. The increase in $CO_2$ in the blood was responsible for a respiratory acidosis with consequent iperpnea-vasodilation, sweating, dehydration, peripheral venous stasis, ispissatio sanguinis, red cell lung clots, and cardiorespiratory failure that led to the death the subject.

**Asphyxia, Confinement, Kidnapping**

## G97 50 Years Later: How Insect Evidence is Key in Turning Over a Wrongful Conviction in Canada's Most Notorious Case – *Regina v. Steven Truscott*

*Sherah L. Van Laerhoven, PhD\*, University of Windsor, Department of Biology, 401 Sunset Avenue, Room 119 Bio, Windsor, ON N9B 3P4, CANADA; and Richard W. Merritt, PhD, and Ryan K. Kimbirauskas, MS, Department of Entomology, 243 Natural Science Building,Michigan State University, East Lansing, MI 48824*

After attending this presentation, attendees will understand the history and cultural impact of R. v. Truscott, and the evidence that lead to overturning this miscarriage of justice.

This presentation will impact the forensic community by illustrating the use of modern evidence in reanalysis of older cases, the importance of having forensic analyses based on scientific evidence and value of recreation experiments.

The body of 12-year-old Lynne Harper was discovered at 1:50 p.m. on June 11, 1959 in a woodlot northeast of Clinton, Ontario. She had been raped and strangled using her blouse. Insect evidence was photographed and collected both at the scene and autopsy, and the insects were reared to adult for identification. However, the insect evidence was not used in the 1959 trial or 1960 appeal. Stomach content analysis was used to pinpoint a 45 minute period for the time of death, two days prior (7:00 – 7:45 p.m., June 9, 1959). Based predominantly on this time frame and some circumstantial evidence, Lynne's classmate, 14-year-old Steven Truscott was convicted of her murder and scheduled to be hanged on December, 1959. A temporary reprieve on November 20, 1959 postponed his execution and on January 22, 1960, his death sentence was commuted to life imprisonment. Truscott was the youngest person to be sentenced to death in Canada, and his case provided the major impetus toward abolition of the death penalty in Canada. Truscott always maintained his innocence. After serving his sentence, Truscott was released and in 2001, he filed for review of his 1959 murder conviction. Fresh evidence was presented at hearings held at the Ontario Court of Appeal in 2006-2007. This new evidence included testimony of three forensic entomologists, with three other forensic entomologists filing reports (but not called to testify) on the insect evidence. Based on the analysis of the insect evidence, a recreation experiment of insect evidence and a reanalysis of the pathology evidence on stomach content analysis, the original estimate of time of death was considered to be unreliable. Truscott was with numerous witnesses prior to 1900 h and after 8:00 p.m. on June 9, 1959, thus the estimate of time of death was the most critical evidence in the original 1959 trial and the 2006-2007 appeal. In 2007, his conviction was declared a miscarriage of justice and Truscott was acquitted of the murder.

**Forensic Entomology, Wrongful Conviction, Historical Cases**

## G98 First Insect Succession Study on a Human Cadaver in Texas

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After attending this presentation, participants will be introduced to the first succession of insects observed on a human cadaver in Texas.

This presentation will impact the forensic community by providing information on the succession patterns and abundances of forensically important insect species attracted to a human cadaver.

These data can potentially be used to determine a more refined estimate of the period of insect activity (PIA) on human remains discovered in the southwestern United States. Correctly identifying arthropod species found associated with a body allows for time approximations to be made based on development data and behaviors. The PIA is divided into two ecological phases termed the pre-colonization (pre-CI) and post-colonization (post-CI) intervals. Insects arrive at bodies in waves or seres. The pre-colonization interval (pre-CI) is defined by insects being initially attracted to remains without colonizing the resource. Various species arrive at different times based on seasonality and abiotic factors. Insects are attracted to a body in a predictable pattern based on its stage of decomposition. Many of these insects will colonize (post-CI) the resource once the remains are discovered. Succession patterns may be influenced by the condition of the body and the area in which it is located. The following factors about the condition of the body may influence the rate of colonization, species richness, and abundance: direct sunlight, partially shaded, indoors, urban, rural, buried, or submerged. Applying the knowledge of arrival time (pre-CI), colonization patterns (post-CI), and associated behaviors allows for a better assessment to be made concerning approximations in the length of time a body may have been at a specific location.

Blow flies (Diptera: Calliphoridae) were initial colonizers of the human remains in this study. Over the duration of this study three blow fly species were regularly collected in the vicinity of the body: *Cochliomyia macellaria*, *Chrysomya rufifacies*, and *Phormia regina*. There were also *Piophilidae casei*, as well as Muscidae and Sarcophagidae species collected from the body. Coleoptera species also will colonize a body because of the readily available food resource of dipteran larvae as well as decomposing materials. Five beetle families were collected near or around the body: Cleridae, Histeridae, Silphidae, Staphylinidae, and Dermestidae.

*C. macellaria* is one of the initial colonizers in warmer temperatures while *P. regina* is more active during cooler weather. The diversity of flies collected may be an indicator of the range of temperatures experienced during the study. The hairy blow fly larva, *C. rufifacies*, is a facultative predator and will feed on larvae of previous colonizers such as *C. macellaria*. The hairy maggot blow fly can be distinguished from other maggots on a resource by the spine-like projects on each segment. It was interesting to note that there was a delayed colonization of Calliphoridae. Abiotic factors such as temperature may have influenced the colonization times or other unaccounted factors may have influenced the delay of oviposition but it is important to note that the body was not immediately colonized. Various ants from the family Formicidae and fleas, *Xenopsylla cheopis,* were also collected during this study. Postmortem ant bites on a body have been previously documented in other studies. Fleas collected near the body do not imply that the subject was infested; rather it may represent the environmental fauna and the potential pests they carry. This study appears to have followed known succession patterns expected for the arthropod species collected; however, this is the first study in the state of Texas to examine insect succession patterns using a human cadaver.

This study is also important because it allows forensic entomologists to better assess delay in colonization estimations of insect activity on remains as it relates to the pre-CI. The specific time when a body is placed out into the field can be compared to estimations based on entomological development data. Establishing the accuracy of time estimations using development data for those insects collected from the remains may lead to more refined methods for calculating how long a body has been in a particular area prior to colonization. It is noted that this is a primary study with many more conditions to be replicated as potential body recovery sites and thoroughly analyzed to gain a better understanding of attraction, colonization time, development, and interactions among insect species on a human resource.

**Insect Succession, Human Cadaver, Texas**

## G99 Comparison of Biological Sensors to Detect Human Remains: Canine Versus Hymenopteran

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After attending this presentation, attendees will have a greater understanding of principle of associative learning and its use to train vertebrate and invertebrate species to detect human remains in unknown samples.

This presentation will impact the forensic community by providing information on the use of canines and conditioned insects to screen soil samples for the presence of human remains.

A tremendous amount of effort in the scientific community has focused on deciphering how animals, as well as plants, receive and interpret environmental stimuli. In regards to forensics, these efforts have primarily targeted the development of biological sensors, such as canines, for tracking missing individuals or escapees from custody. Other efforts have evaluated the use of canines to detect explosives or narcotics in places frequented by people.

In recent years the U.S. Department of Defense has initiated research examining the ability of arthropods to detect and locate compounds of human importance. *Microplitis croceipes* has served as a model for a number of studies to detect plant pathogens, explosives, and human remains (Lewis & Martin, 1990; Takasu & Lewis, 1993, 1995, 1996; Rains 2004, 2006). These efforts have translated into the development of a biological sensor that is capable of detection at the nanogram level.

While the use of cadaver dogs in detecting human remains is widely accepted, there is little research that scientifically validates the capabilities or mechanisms by which the dogs function. This study compared cadaver dog performance to that of the trained wasps in terms of threshold and accuracy. Five nationally certified and experienced cadaver dogs with "real world finds" were tested in two types of evaluation paradigms. The cadaver dogs varied in age from three to nine years and consisted of two Border Collies, one Rottweiler, one Laborador Retriever, and one Doberman Pinscher. Cadaver dog threshold was equal to *M. croceipes* as the dogs were able to detect and alert to the presence of human remains using 0.02 grams of soil from beneath a human cadaver mixed with 20 grams of background soil.

Two types of presentation trials were utilized. The first trial set consisted of singular jar presentation in a room. This matched the presentation to the conditioned wasps but was not a traditional method of training or evaluation for testing detector dogs. The second trial, which was conducted upon the end of the first trial, consisted of a scent line-up with all four targets present. Targets were placed approximately three feet apart in a single row line. This is a traditional presentation utilized in research and training of scent dogs.

No type one errors were seen with the dogs; however, there were type II errors. Training biases may account for some of the error margin. Target odors are most often placed into containers for preserving and storing the target for continued use. Preservation of training aids necessitates the use of containers, cages, and other devices which can subsequently become a visual cue for the dog. Most research performed on scent detection dogs involve line ups or concealed target odors to avoid visual cueing. Placing containers in plain sight may have lead to a bias based on expectations by the dog's previous experience. Cadaver dog training scenarios typically include at least one target odor within a designated search area; therefore the dog is expecting to find something. This defines a need for cadaver handlers to continue to train their canine partners on scenarios involving visual negative targets to reduce association between a visual target and alerting, thereby increasing their efficiency on real world searches.

Cross contamination, residual scent, indoor ventilation systems, and container placement may have also contributed to the type II errors. Cross contamination can also occur due to residual scent. A recent study indicated that dogs can detect human remains odors of human corpses on carpet squares even though the squares did not come into direct contact with the corpse (Oesterhelweg, 2008). Since so little is still known about detector dogs, research that help define thresholds and other factors is essential in increasing the effectiveness of these dogs.

**Canine, Microplitis Croceipes, Biological Sensor**

## G100  Generating Development Data for Forensically Important Flies That Are Difficult to Rear in the Laboratory

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After attending this presentation, attendees will learn how carrion fly development data can be obtained for species that are not suitable for rearing under common laboratory methods.

This presentation will impact the forensic community by providing rearing techniques for blow flies that have minimal development data due to their difficulty to rear in a laboratory setting. With these data forensic entomologist can generate more accurate development data for commonly encountered forensically important blow flies leading to increased accuracy and precision in postmortem interval estimations.

Forensic entomologists have so far been unsuccessful in their attempts to establish laboratory colonies of some of the more commonly encountered carrion insect species. Therefore, it has been difficult to produce the growth models often used to estimate a minimum postmortem interval based on specimen age of these species. Notable examples of this technical problem in North America are the green bottle flies *Lucilia illustris* and *Lucilia coeruleiviridis*. It has been found that a wild-caught *L. illustris* female will lay eggs in a laboratory cage, but the resulting f1 generation will not mate under these standard rearing conditions. *Lucilia coeruleiviridis* presents an even more difficult problem, in that post-feeding *L. coeruleiviridis* larvae, either collected from a corpse or obtained from a wild adult female, will not pupate under laboratory conditions. The larvae have been observed to go into an extended wandering stage lasting several days only to eventually shrivel and die.

It was hypothesized that post-feeding *L. coeruleiviridis* larvae require a larger pupation medium volume than is typically used for laboratory culture. The hypothesis that rearing *L. coeruleiviridis* eggs obtained from wild females in containers much larger than those used in this research would have an effect on their development was tested. *Lucilia illustris* was similarly investigated because of the lack of developmental data currently available for this species as well.

During midsummer at the study site in northwest Indiana, 11 piglet carcasses were exposed to short duration (max 2.5hrs) fly activity and inspected for eggs every half hour. Once eggs were observed, the piglets were individually placed on (approximately $0.11 \text{ m}^3$) leaf litter, collected on site, in large plastic storage tubs (approximately $.16 \text{ m}^3$). Breathable cloth-like material was immediately secured over the plastic tubs to exclude further oviposition. Ambient (outside) and interior container temperatures were monitored. Adult flies that emerged from a container were collected daily and identified.

Aspects of development will be discussed for three of the blow flies that were successfully reared to adulthood in this experiment; *L. illustris*, *Lucilia sericata*, and *L. coeruleiviridis*. Physiological time calculations for *L. illustris* were compared to those reported by other authors. Both *L. coeruleiviridis* and *L. illustris* developmental data were contrasted to the extensively studied *L. sericata*, because some investigators have used *L. sericata* growth models to estimate *L. coeruleiviridis* age. Under these conditions *L. coeruleiviridis* total development time was numerically longer than that of *L. sericata*. Total development time of *L. illustris* was comparable but slightly more accelerated than that of *L. sericata*.

The procedure used in this experiment provides forensic entomologists with a means of obtaining growth rate data for flies that were previously difficult to rear. Having data on these forensically important flies can be used to increase precision and accuracy of estimations of the postmortem interval.

**Lucilia, Postmortem Interval, Forensic Entomology**

## G101  Insect Pupal Cases as Decay-Resistant Reservoirs of Human Soft Tissue Radiocarbon Content

*Gregory W. Hodgins, DPhil\*, University of Arizona, Department of Physics, 1118 East Fourth Street, Tucson, AZ 85721*

After attending this presentation, attendees will understand how above-ground nuclear testing in the 1950s and early 1960s dramatically increased environmental levels of radiocarbon. These elevated levels have been incorporated into all organisms living since that time and thus can serve as temporal markers. Potentially, radiocarbon measurements of postmortem human tissues can be used forensically to establish year-of-birth and/or year-of-death. One advantage of this approach is that it functions independently of chemical or biological methods for the determination of postmortem interval or age-at-death and thus might augment current methods for establishing these parameters.

This presentation will impact the forensic community by outlining the possibility of determining year-of-death of human remains in advanced stages of decay based on the radiocarbon content of insect pupal cases obtained from the surrounding soil. Remains in advanced stages of decay pose particular challenges for determining postmortem

interval, and other temporal parameters. The presentation has two objectives: (1) to outline a hypothesis and an experimental design, and (2) to canvas the forensic science community for suitable samples.

Over the past sixty years, environmental levels of radiocarbon have been rapidly changing. Previous work in this laboratory has established that radiocarbon levels in human soft tissues essentially reflect levels in the contemporary environment. Therefore measuring radiocarbon levels in postmortem tissues and correlating these with known levels in the past environment can indicate Year-of-Death. Direct measurements on tissues from known age/known year-of-death donors have shown promise and quantified the potential precision of this approach to approximately ± 2.5 years.

The paradox of suggesting such an approach is that in many environments, soft tissues disappear within short spans of time due to natural process of decay. What are required are decay-resistant proxies of soft tissue radiocarbon content. The hypothesis of this study is insect pupal cases might fit the bill. Just as humans take on the radiocarbon content of ingested foods, insect larvae feeding on decaying human remains take on the remains' radiocarbon content. Although emergent adult insects leave, pupal cases are left behind. Large numbers are often encountered in the soil surrounding decayed remains long after soft tissues have disappeared.

Preliminary measurements on samples generated from field tests will be presented as well as measurements on paired samples of soft tissues and pupal cases obtained from a Medical Examiner's Office archives. The design of future experiments will be discussed.

Admittedly, the approach is potentially complex. For example, direct measurements of radiocarbon levels in different human tissues show tissue-specific variation. These differences are the consequence of rapidly changing environmental levels and differences in metabolic turnover rates. Consequently, one might expect that insect larvae feeding on different tissues of the same individual might be differentially labeled. On the one hand, this might reduce the precision of Year-of-Death estimations. However, if species-specific differences in larvae feeding behaviors exist among the succession of insects that infest decaying remains, this might result in species-specific differences in pupae radiocarbon levels. Such differences might be exploited to advantage. Clearly experimental data is required.

The approach is intriguing. It would require a trivial modification of existing sample collection practice: merely collecting a larger than normal number of pupae. It is potentially a new avenue for the forensic estimation of Year-of-Death.

**Radiocarbon, Year-of-Death, Pupal Cases**

## G102  Reconstruction of Decay Processes of a Dead Child's Body in a Plastic Garbage Bag

*Heike Klotzbach, PhD\*, Institute of Legal Medicine, Fürstengraben 23, D-07743 Jena, GERMANY*

After attending this presentation, attendees will know how important questions concerning a homicide case were answered by step-by-step reconstruction using pig cadavers and special knowledge of various disciplines.

This presentation will impact the forensic community by underlining the statement that "extraordinary" methods are sometimes helpful in forensic taphonomy and moreover emphasize the cooperation of scientists form different disciplines.

The remains of a 10-year-old girl hidden in a plastic garbage bag in a wooded area in Northern Germany were recovered. The girl had been reported missing three months earlier. Her body was in a stage of advanced decay, the soft tissue had been entirely liquefied, colored grayish pink and foamy in sections, whereas the bare skeleton was visible. The bones were complete and the arrangement of the skeleton indicated that the body had originally been left in *anteflexion* with bent knees. The foot bones were still inside the shoes and the underpants were positioned around both ankles indicating preceding sexual abuse. No evidence as to mammalian-feeding defects neither to preceding blow fly (Diptera: Calliphoridae) activity was observed. Only some skipper flies (Diptera: *Piophilidae*, "Cheese Skippers"), well known as late colonizers, were detected. Considering the climate of northern Germany the question had to be answered. Could a dead body get into that advanced state of decay within three months time without addition of any chemical substances? Extensive toxicological investigations merely resulted in high concentrations of calcium (1120 mg/kg) in the liquefied tissue. The influence of hydrogen peroxide was considered but excluded because large quantities of the long scalp hair were found still in its original brown color.

Experiments with pig cadavers (n=14; 20-30 kg) in plastic bags under equivalent environmental conditions revealed that soft tissue was liquefied equally with the skeleton left in anatomical position in those cases without addition of any chemical substances (e.g., quick lime). The pig cadavers where quick lime was added (1:3, 1:10, 1:30) were found with rather dry and hard soft tissue, aridity was increasing with the concentration of quick lime. By following investigations in a specialized microbiological laboratory *Clostridia* species (*C. limosum, C. novyi, C. sordellii, C. sporogenes*) were detected in the remains of the child as well in the liquefied porcine tissue. These *Clostridia* species are reported to produce both histolytic and cytolytic enzymes. Also the foamy consistency of the soft tissue could be explained due to the well documented gas producing activity of *Clostridia* species. Furthermore high calcium levels equivalent to these in the original specimens were determined in the liquefied porcine tissue.

In summary it was concluded, that the body of the child inside the plastic bag reached the state of liquefaction without addition of any chemical substances. The environment inside the closed plastic bag without oxygen supply promoted a shift to benefit the development of the anaerobe bacteria like *Clostridia* species. Hence high concentrations of histolytic and cytololytic enzymes secreted by these microorganisms resulted in a relatively fast liquefaction of the soft tissue. These conclusions were also in accordance with the crime scene analysis (closed plastic bag above ground, underpants around ankles of the corpse) indicating a fast disposal of the dead body after sexual abuse.

**Decay Processes, Plastic Bag, Taphonomy**

## G103  Unusual Methods of Suicide in Chicago, Illinois, Cook County

*Michelle A. Jorden, MD\*, James A. Filkins, MD, JD, PhD, and Tera A. Jones, MD, Cook County Medical Examiner Office, 2121 West Harrison Street, Chicago, IL 60612*

After attending this presentation, attendees will lean about some unusual methods of suicide recently observed in Chicago, IL during the first half of 2008. This paper is being introduced during a time of economic strain within the United States.

This presentation will impact the forensic community by making attendees aware of the unusual methods of suicide are being observed in Chicago, IL.

In 2004, according to the National Institutes of Health (NIH), suicide was the eleventh (11th) leading cause of death in the United States, accounting for 32,439 deaths. The major risk factors for the commission of suicide are well known and include a history of depression, substance abuse, stressful life events, family history of suicide, and prior suicide attempt. Males are four times (4X) more likely to commit suicide compared to females. Recent research has suggested

the risk of suicide may be the result of an imbalance of neurotransmitters in the brain, thus emphasizing the importance of diagnosis and the role of antidepressants in the treatment of depression.

Firearms, suffocation, and poisoning were the most common methods chosen by individuals to commit suicide, although the methods differed between the sexes. Males tend to commit suicide using firearms, whereas females commit suicide by poisons. Non-Hispanic whites commit suicide at the highest rate. Although some data is similar to that published by the NIH, the authors will introduce a total of eight individuals who committed suicide by unusual methods rarely seen in a major metropolitan area.

One case involves a white male with multiple shotgun wounds who was found in his secure residence. The second case involves a white male inflicting sharp force injury to his dialysis catheter causing exsanguination and air embolism. Two cases, a white male and white female, involved the "death by Hibachi" method, which is accomplished by carbon monoxide intoxication from burning charcoal in an enclosed environment. At autopsy, both cases revealed bright cherry-red lividity and the carbon monoxide level ranged from 54%-80% saturation. One case involved a white female chemistry student who ingested acetylferrocene, an orange crystalline powder that is extremely toxic once ingested, and who died of liver failure. One case involved a white male hanged with simultaneous electrocution from a manmade apparatus. One case involved a white male who used ligature strangulation as a means of suicide. Finally, the last case involving a white male is noteworthy and unusual in the sense that the commission of suicide was performed with the production of hydrogen sulfide gas. This is a most unusual case of suicide from medical examiners office but deserves mention as this method is becoming increasingly popular overseas.

Suicide notes were left at the scene in only three cases, a similar frequency seen in prior reports. Two detailed suicide notes were recovered from individuals performing the "death by Hibachi" method. The third suicide note was recovered from the male inflicting sharp force injury to his dialysis catheter.

With the introduction of the internet, old as well as new and more unusual methods of committing suicide are available to the population. As seen in this research, the "death by Hibachi" method would take time to plan and execute (i.e., spending time on the internet, buying and burning the charcoal, taping the doors in the room and writing detailed suicide notes).

All cases involved non-Hispanic white individuals, similar to that seen in the NIH data. In this study, there was a preponderance of males committing suicide (6:2). In each of the cases, the reasons for committing suicide coincide with the NIH data (i.e. depression, stressful life events). Although one of the cases involved a male using a firearm as a means of suicide and one case involved a female using a poison to commit suicide, these were not common means of suicide as people seldom kill themselves by inflicting multiple shotgun wounds and ingesting acetylferrocene. Additionally, people seldom commit suicide via means of ligature strangulation. The remaining cases also illustrate uncommon and unusual methods of suicide. In this small study, six of the eight individuals had a prior documented suicide attempt.

Suicide is one of the most preventable deaths in the society and the recognition and treatment of depression is underscored. However, the medical examiner/coroner will continue to examine suicide deaths especially in economic hardship as recently experienced in the United States. This paper serves to introduce some uncommon methods of suicide recently observed during the 2008 year.

**Suicide, Unusual Methods, Chicago, IL**

## G104 Mass Fatality Investigation Due to Combustible Dust Related Industrial Explosion and Fire

*J.C. Upshaw Downs, MD\*, and Edmund R. Donoghue, MD, Regional Medical Examiner, Georgia Bureau Investigation, 925 A Mohawk Street, Savannah, GA 31419-1796*

After attending this presentation, the attendee will recognize the dangers of combustible dusts and their relation to industrial deaths, and better understand unique features of mass fatality investigation in an industrial setting and an active fire scene.

This presentation will impact the forensic community by exposing practitioners to the under-recognized dangers of combustible dusts and the complexities involved in mass fatality in an industrial/fire setting.

Combustible dust is an under-recognized industrial hazard. The United States Chemical Safety Board identified over 280 events with 837 casualties (including 119 deaths) in the period 1980 to 2005. The industries involved are varied and include organic dusts (wood, sugar, grains, etc.), metal powders (magnesium, aluminum), chemical manufacturing, plastic production, pharmaceutical production, and coal handling/processing. In fact, "any industrial process that reduces a combustible material and some normally noncombustible materials to a finely divided state presents a potential for a serious fire or explosion" (NFPA's Industrial Fire Hazards Handbook). In fact, sugar may seem harmless but is recognized as a strong explosion hazard (Bureau of Mines – "The Explosibility of Agricultural Dust"). In addition to the usual fire-triangle components (fuel, fire, and oxygen), a combustible dust explosion requires sufficient quantity and concentration of dust in a confined space. A major risk in such settings is the rapid dispersion of previously quiescent depots of dust particles follows a lesser primary explosion. With a significant fuel reservoir abruptly literally shaken loose and into the ambient air, a more devastating secondary explosion can be anticipated if the reservoir ignites. Safety procedures can reduce the risks associated with combustible dusts, especially related to the fuel, dispersion, and ignition but are less effective in controlling the confinement and ambient oxygen.

Shortly after 9 p.m., a series of explosions rocked the second largest cane sugar refineries in the United States (responsible for ~15% of total national production). The fires took days to extinguish due to the nature of the incident – a large depot of molten sugar remained ablaze in one of three storage silos despite significant efforts to put it out. Up to an estimated 100 personnel (of 472 total) were reported working in the affected plant area at the time of the blast. Of these, upwards of 40 individuals were seriously injured and a total of eight individuals were eventually reported missing and presumed dead. Recovery efforts, including law enforcement and medical examiner staff, to locate the presumed deceased proceeded while the silo fire was actively burning and scene stability was questionable. Over the ensuing days and weeks, the bodies of the dead were recovered and identified. The extensive thermal damage to those who remained in the burning plant longest posed identification challenges due to fragmentation and calcination. At the conclusion of the medicolegal investigation, all eight dead on scene were identified and the remains were re-associated with the appropriate individual. An additional five fatalities occurred related to extensive burn injuries, for a total 13 deaths. The case resulted in extensive media scrutiny and eventually the third-largest fines in OSHA history.

This presentation reviews the nature and dangers of combustible dust related fires. Specific issues related to the death investigation process and body recovery are addressed. The investigative outcomes, including recognized risks and identified cause are presented.

**Combustible dust, Explosion, Sugar**

## G105  Worker Fatalities by Hydrogen Sulfide Poisoning:  Autopsy and Toxicological Findings

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After attending this presentation, attendees will have an understanding of some particular aspects of Hydrogen Sulfide poisoning which is an important cause of work-related death.

This presentation will impact the forensic community by emphasizing the fact that Hydrogen Sulfide (H2S) is a harmful and lethal chemical, and accidents may occur upon exposure to it in its natural gaseous state in various work environments.

The goal of this presentation is to recount the story of the deaths of five men who, while working in a truck tank which transported liquid sulfur, were poisoned by Hydrogen Sulfide.  Variations in pathological and histological findings, coupled with toxicological results, and crime scene investigations will be illustrated.

Hydrogen Sulfide is a powerful, rapidly acting, colorless, poisonous gas.  $H_2S$ has a specific gravity (1.19) higher than air, and its presence can be detected by its characteristic odor of rotten eggs.  Acute occupational poisoning and fatalities have been reported from exposure to $H_2S$ in industrial settings, sewage disposal facilities, and septic tanks.   This gas is very unstable and thiosulfate is its major metabolized substance.  For this reason, the presence of thiosulfate is known to be a useful indicator of Hydrogen Sulfide poisoning in forensic analysis.

**Case History:**  Five workers were found motionless in an empty truck tank which had previously contained liquid sulfur.  They were soon removed from the tanker; four of the five men had already died.  The fifth man, who was also the youngest, died at the hospital the following day.  Crime scene investigation revealed that the first of the victims began the cleaning operation of the truck tank when he became unconscious.  One by one, fellow colleagues attempted to rescue their co-workers, each succumbing to the toxic gas, and each falling into unconsciousness, ultimately followed by death

**Autopsy Findings:**  The workers had a mean age of 37.6 years (range 20-64).  External examination of the bodies revealed congestion of the head, neck, and shoulders with cyanosis of lips and fingernails in all cases.  The ocular conjunctiva showed marked hyperemia and a few petechiae.  Two workers displayed traces of solid yellow sulfur on their faces and on the soles of their shoes.  One of the workers, who was 23-years-old, displayed a very characteristic greenish discoloration of his eyes, anterior cervical region, and precordia.  Only one worker showed signs of putrefaction.  Two of the men presented with blunt force injuries on the occipital areas with subgaleal contusions which resulted from falling.

Upon internal examination, it was noted that the lungs of all the workers were heavy with edema and congestion which was also present in the kidneys and spleen.  The 23-year-old worker displayed a greenish discoloration both in the thorax muscles, as well as on the surface of the stomach.  There were no remarkable findings related to the other organs except for slight cerebral edema which was present in all five victims. In addition, an aortocoronary bypass graft was present in the oldest victim. Microscopic examination revealed passive congestion which was evident in the lungs, spleen, kidneys, and adrenal glands.  Massive hemorrhagic edemas were found in all the workers, most notably in the youngest victim who died 12 hours after the tragic event.

*Toxicological results*:  Toxicological analyses of peripheral blood vessels (femoral) were negative for alcohol and illicit drugs in all of the workers.  Thiosulfate in the heart blood was quantified using a gas chromatography-mass spectrometry (GC/MS) technique after derivatization with pentafluorobenzyl bromide.  Each of the victims had a blood thiosulfate level that, according to other international reports, was enough to determine that the cause of death was due to fatal hydrogen sulfide poisoning: thiosulfate levels ranged from 2.6 mg/l (first worker in who entered into the tank) to 183 mg/l.

The analyses performed on air samples collected from inside the truck tank, as well as analogous trunk tanks used for liquid sulfur transport revealed that $H_2S$ air concentration levels were high to have caused these occupational fatalities.

**Hydrogen Sulfide, Worker Fatalities, Thiosulfate**

## G106  Suicide by Hanging in Harris County, Texas

*Sara Nunez-Doyle, MD\*, and  Sharon M. Derrick, PhD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will have comprehensively reviewed the features of suicidal hangings, including demographic characteristics of the decedents, frequency and types of injuries identified at autopsy, ligature characteristics, and scene investigation information.  This review will provide the attendee with a reference base for common and uncommon findings in suicidal hangings.

This presentation will impact the forensic community by providing insight into the case findings and epidemiological characteristics of suicidal hangings presenting to the Harris County Medical Examiner's Office from the large diverse population of Harris County, Texas.

Suicidal hangings comprise 19% (320) of the 1676 suicides that occurred in Harris County, Texas from January 1, 2004, through June 30, 2008.  Hanging was the second most common method of suicide after firearm wounds.  An upward trend in the total number of hangings was recorded over this four and one-half year period, with 51 hangings occurring in 2004, 73 in 2005, 71 in 2006, 81 in 2007, and 43 in the first half of 2008.  Although the total number of suicides in Harris County also increased over this period (from 298 in 2004 to 424 in 2007), the percentage of hangings compared to total suicides increased, with hangings comprising 17.1% of the total suicides in 2004 and 21.8% of the total suicides in the first half of 2008.  Accounting for the approximated 7% population increase in Harris County from 2004 through 2007, the actual rate of suicidal hangings increased slightly over this period, from 1.3 per 100,000 people in 2004 to 2.1 per 100,000 in 2007, as did the rate of total suicides, from 8.1 per 100,000 in 2004 to 10.8 per 100,000 in 2007.

The majority of the decedents who hanged themselves (81%) were male, a trend that is common for other methods of suicide.  Female decedents comprised 19% of the suicidal hangings and 24% of the total number of suicides in the period examined.  The ages of decedents hanging themselves over the period studied ranged from 10 to 80. Children were over-represented in the hanging category when compared to total suicides.  Ten percent of the decedents hanging themselves were under the age of 18, compared to 3.8% of the decedents committing suicide by all methods.   Senior citizens were under-represented. Decedents over the age of 65 comprised 2.5% of suicidal hangings compared to 11.5% of all suicides in the time period studied.

The majority of decedents who hanged themselves were Caucasion (54.4%), followed by Hispanic persons (31%), African-American persons (10.1%), and persons of other race/ethnicity (4.5 %).  The breakdown of ethnicity for people dying by suicide by all methods was

similar, with 68% white, 18% Hispanic, 11% black, and 3% other race/ethnicity. In suicidal hangings as well as suicide by all methods, white persons appear to be over-represented when compared with the population breakdown of Harris County, in which approximately 37% of the population is white.

Of the 320 suicidal hangings examined, 260 (81%) took place in the decedent's residence or property immediately surrounding the residence (yards, garages, utility sheds, and other outbuildings). Of the remaining 19% of cases, the more common locations of the hangings included parks, fields, or wooded areas (15 cases), jails or other correctional facilities (13 cases), places of business (11 cases), and motels or hotels (8 cases). Hanging was the only method of suicide used by incarcerated persons over the four and one-half year period studied. Approximately one-fifth of the decedents (62 cases) were transported from the scene of the hanging and received medical care prior to being pronounced dead. The remaining four-fifths of the decedents were pronounced dead at the scene of the hanging.

The types and frequency of injuries of the internal neck structures identified at autopsy such as hemorrhage of the neck musculature, fractures of the hyoid bone, and fractures of the tracheal and laryngeal cartilages as well as injuries of the spine will be reviewed in detail. In addition, the various types of ligatures recovered, ligature positioning, and positions in which the decedents hanged themselves will be discussed. The prevalence of factors such as previously diagnosed mental illness, physical illness, and prior suicide attempts in decedents who hanged themselves will also be examined.

**Hanging, Suicide, Epidemiology**

## G107 Death in a Tanker Truck

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After attending this presentation, attendees will understand the need for thorough investigation of work-related fatalities and will be familiar with the autopsy presentation of death by inhalation of caustic substances.

This presentation will impact the forensic community by suggesting strategies for investigating occupational deaths by using interagency communication and cooperation.

In this case, a 23-year-old man was cleaning the inside of a tanker truck which had been used to carry 50% potassium hydroxide. The procedure for cleaning the tank included a confined space entry procedure, consisting of 21 pages of instructions.

The cleaner used spray head to flush the tank with water at least four times, then used a fan to dry the tank. The tank would be checked for adequate oxygen concentration using a digital meter. A worker would then go into the tank, using a safety harness, mask, ladder, personal air monitor, personal motion detector, and protective equipment. A second worker, acting as the safety attendant, remained outside the tank. Ventilation was introduced by using an air hose inserted into a small hatch in the tank. The worker would remove any remaining chemical using a high-pressure water hose, hand dry the tank, and inspect it for corrosion. During the cleaning procedure, the truck engine was off.

On the day this case occurred, the decedent was working alone. Fifteen minutes after he entered the tank, another worker checked on him and found him unresponsive at the bottom of the tank. He was removed from the tank using his safety harness, and taken to a hospital, where he was pronounced dead.

Autopsy showed pulmonary edema, and pulmonary and gastric hemorrhage. Toxicology was negative except for vitreous urea nitrogen of 30 mg/dL.

The supervising Coroner's investigator and Chief Medical Examiner-Coroner visited the scene and discussed the procedures used for cleaning the tank with employees of the transportation company. In addition, Coroner's staff met with the involved police agency and representatives of California Department of Industrial Relations, Division of Occupational Safety and Health. The Material Safety Data Sheet for potassium hydroxide and the medical literature provided additional information.

The cause of death was determined to be pulmonary edema due to potassium hydroxide exposure and other undetermined factors. Dehydration was given as a contributing condition.

The medical literature contains reports of chronic obstructive lung disease following sodium hydroxide inhalation. However, rapid death after potassium hydroxide inhalation has not been reported. The mechanism of death proposed for this case is that the caustic potassium hydroxide produced widespread pulmonary edema, with rapid degradation of respiratory function. The exothermic reaction of potassium hydroxide and water may have contributed to death by heating the inside of the tank.

Given the non-specific autopsy and toxicology findings, additional investigation was essential in determining the cause of death for this decedent. Through cooperation between the Coroner, law enforcement, and occupational health agencies, the cause of death could be established in this case.

**Occupational Health, Potassium Hydroxide, Inhalation**

## G108 Agonal Sequences in Eight Filmed Hangings: Analysis of Respiratory and Movement Responses to Asphyxia by Hanging

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After attending this presentation, attendees will have a better understanding of the pathophysiology of human asphyxia by hanging.

This presentation will impact the forensic community by providing new insights into the body responses to asphyxia by hanging, based on results from the Working Group on Human Asphyxia.

**Introduction:** In the conducting of investigations and trials, forensic pathologists are often asked questions related to body responses in human asphyxia. Those questions are very difficult to answer considering the paucity of literature. Animal studies have been conducted, but the extent to which those results can be applied to human is doubtful. As for direct human experimentation, it is of course out of question for obvious ethical concerns. To palliate these limitations, the Working Group on Human Asphyxia was formed in 2006 at the 58th Meeting of the AAFS in Seattle. This working group has for main objective to regroup filmed hangings in order to give new insights into the pathophysiology of human hanging.

**Methods:** A total of eight filmed hangings from three different countries (Canada, Switzerland, and United-States) were analyzed: two filmed suicides and six autoerotic deaths. Hangings were of different types: free hanging, hangings with feet on the ground, hanging kneeling and hanging almost lying face-down. The hanging ligatures also varied widely, from cloth band to ropes with or without padding and electric

cords. All victims were adult males. Those filmed hangings were compared in terms of loss of consciousness, convulsions, decortication and decerebration rigidities, loss of muscle tone, last muscle movement, and respiratory responses. The time frame at which each of these responses occurred was taken by two judges.

**Results:** With the time 0 representing the onset of hanging, rapid loss of consciousness was observed (at 8 – 18 seconds), closely followed by appearance of convulsions (at 10 – 19 seconds) in all cases. A complex pattern of decerebration and decortication rigidity was then observed in all cases. Last isolated muscle movement occurred between 1 minute-2 seconds and 7 minutes-31 seconds. High similitude was observed for respiratory responses: onset of very deep respiratory attempts between 13 and 24 seconds, last attempt between 1 minute-02 seconds and 2 minutes-05 seconds.

nd : no data/– not observed

| | Case #1 | Case #2 | Case #3 | Case #4 | Case #5 | Case #6 | Case #7 | Case #8 |
|---|---|---|---|---|---|---|---|---|
| **Movement Responses** | | | | | | | | |
| Loss of consciousness | 13s | nd | 18s | nd | 10s | 8s | 10s | 12s |
| Convulsions | 15s | 14s | 19s | 18s | 13s | 11s | 10s | 14s |
| Decerebration | 46s | 19s | 21s | nd | 1min19s | 31s | 11s | 20s |
| Decortication #1 | 21s | 1min08s | 1min00s | nd | 59s | 33s | 26s | 31s |
| Decortication #2 | 1min11s | 1min32s | 1min04s | nd | – | – | 34s | – |
| Loss of muscle tone | 1min38s | 2min47s | 2min04s | nd | 1min52s | – | – | – |
| Last muscle movement | 4min10s | 3min01s | 3min01s | nd | 7min31s | 1min02s | nd | nd |
| **Respiratory Responses – Very Deep Respiratory Attempts** | | | | | | | | |
| Start | 20s | 21s | 22s | 24s | 13s | 19s | 13s | 16s |
| End | 2min00s | 2min47s | 2min04s | nd | 2min5s | 1min02s | nd | nd |

**Conclusion:** Despite differences in the types of hanging, similarities could be revealed regarding rapid loss of consciousness and onset of convulsions, pattern of decortication rigidity and respiratory responses. To date, this is a unique study of agonal movements in asphyxia by hanging. The importance of inter-laboratory collaboration in extending this project by adding other available filmed hangings is discussed and the importance of the Working Group of Human Asphyxia (WGHA) is further emphasized.

**Asphyxia, Hanging, Physiopathology**

## G109 Dead Victim Identification: Age Determination by Analysis of Bomb-Pulse Radiocarbon in Tooth Enamel

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After attending this presentation, attendees will understand how biological samples produced in the past 60 years can be dated using the radiocarbon bomb-pulse. Specifically, they will learn how the 14C content of dental enamel can be used to determine year of birth of persons born after 1945.

This presentation will impact the forensic community by providing a technique that improves the accuracy in age determination of dead victims, such as suspected homicides and victims of mass disasters.

**Background:** Determining the age of an individual is an important step in dead victim identification, particularly in suspected homicide cases and in mass disaster work. Age determination can be performed with high precision up to adolescence by analysis of dentition, but establishing the age of adults has remained difficult. The enamel of individual permanent teeth is formed at distinct, well-characterized time points during childhood. After being laid down, there is no turnover of enamel, so its $^{14}C$ concentration reflects the level in the biosphere at the time of enamel formation. Atmospheric testing of nuclear weapons doubled the global $^{14}CO_2$ level between 1955 and 1963. After adoption of the Partial Test Ban Treaty in 1963, the level of atmospheric $^{14}CO_2$ started to decrease exponentially with a mean life of about 16 years due to transport into large carbon reservoirs such as the oceans and losses to space. The enhanced level of $^{14}C$ worked its way up the food chain from $CO_2$ so that all living things are labeled with the pulse.

**Material and Methods:** The concentration of $^{14}C$ in tooth enamel from individual teeth and related it to the known concentration in the atmosphere over time (1950 – present) to establish the time of tooth formation was measured. The dates were then used to estimate the year of birth of the person. To this end, the crown of the tooth was cut away from the root at the level of the cervical line. The crown was then immersed in 10N NaOH, before being placed in a water-bath sonicator. The enamel was then washed with $DDH_2O$ and re-submersed in 10N NaOH during approximately four days to remove all dentin until pre-treated for accelerator mass spectrometry (AMS) analysis.

**Results:** The technique matched $^{14}C$ content in enamel to known age very well along the bomb spike curve. The absolute difference between estimated age and true age was $1.1 \pm 0.9$ years for teeth from Scandinavian subjects, implying a much higher precision than any previous method. Analysis of teeth from deceased subjects from other continents showed similar accuracy, suggesting that the geographical variation of the bomb-pulse radiocarbon does not significantly influence the readings. For teeth formed before 1955, the $^{14}C$ analysis can only tell that the person was born before the nuclear tests (birth year of person before 1945 - 1952, depending on type of tooth analyzed), but with absolute certainty.

**Conclusion:** AMS analysis of teeth offers a precise age determination that can be applied in forensic casework, particularly to assist in investigations of unidentified human cadavers. If radiocarbon determination and aspartic acid racemization analysis of teeth are combined, information of both the year of birth and the year of death can be established.

**Age Determination, Radiocarbon, Tooth**

## G110 Postmortem Injury Detection in an Aviation Mishap: Computed Tomography Imaging Versus Autopsy

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After attending this presentation, attendees will understand the utility and limitations of postmortem computed tomography (CT) analysis of traumatic injury victims compared with standard autopsy.

This presentation will impact the forensic community by demonstrating how CT imaging can complement the conventional autopsy in the injury detection of aviation mishap victims.

**Purpose:** This study compared and contrasted the sensitivity of CT imaging versus autopsy in the postmortem detection of injuries sustained in an aviation mishap.

**Methods and Materials:** Four male victims from an aviation mishap were studied with whole-body CT examinations prior to conventional autopsy. Autopsies were conducted by forensic pathologists and autopsy reports created without reference to the CT

imaging studies. Blinded to autopsy reports, two radiologists then retrospectively interpreted each study in a consensus fashion. Images were evaluated for fractures, dislocations, and soft tissue abnormalities resulting from traumatic injury. Radiology interpretation was compared to autopsy reports to determine the sensitivity of each method in detecting these injuries. CT studies were then re-examined to review missed or discordant findings in order to determine if a successful imaging correlate with the autopsy results could be obtained.

**Results:** Autopsy and CT imaging detected a total of 236 fractures and dislocations. Autopsy detected 139 (59%) and CT imaging detected 231 (98%) of these findings. In regions of the body that were not fully explored during the autopsy procedure (e.g., posterior vertebral body elements, scapula, and ribs), the CT images frequently revealed fractures not recorded on the autopsy reports. Autopsy and CT imaging detected a total of 56 soft tissue abnormalities. Autopsy detected 55 (98%) and CT imaging detected 14 (25%) of these findings. The detailed description of soft tissue abnormalities found in the autopsy reports was frequently not appreciated with CT imaging. Some of these soft tissue abnormalities were apparent in retrospect after being un-blinded to the autopsy reports.

**Conclusion:** The use of CT imaging is a useful adjunct to autopsy in the postmortem detection of injuries following an aviation mishap. CT imaging demonstrates high sensitivity for the detection of fractures and dislocations but is currently limited in the detection of soft tissue injuries.

*The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Army, Department of Defense, or the United States Government.*

**Computed Tomography, Traumatic Injury, Aviation Mishap**

# G111    Usefulness of Systematic Histological Examination in Routine Forensic Autopsy

*Geoffroy Lorin de la Grandmaison, PhD\*, AP-HP, Philippe Charlier, PhD, AP-HP, and Michel Durigon, PhD, AP-HP, 104 Boulevard Raymond Poincaré, Raymond Poincaré Hospital, Garches, F-92380, FRANCE*

After attending this presentation, attendees will be aware of the considerable discrepancy rate between macroscopic and microscopic findings provided by standard histology in forensic autopsy.

This presentation will impact the forensic community by showing that histology is an important feature regarding forensic autopsy quality and is still essential to confirm, refine, or refute macroscopic findings.

**Material and Methods**: A prospective study was carried out on 1,786 autopsies performed in the department of pathology and forensic medicine at the Raymond Poincaré hospital from 2003 to 2007, for which standard histological examination was systematic according to autopsy protocol (including microscopic sections of the heart, lungs, liver, kidneys, pancreas, spleen, thyroid, adrenal glands, prostate and neuropathological study after brain formalin fixation). Histological sections were stained with haematoxylin and eosin. From all these autopsy cases were randomly selected 428 cases for which microscopic sections were reviewed by two forensic pathologists. SIDS cases and skeleton cases were excluded from the study. For each case, information provided by histology regarding respectively cause and manner of death, death mechanism, prior medical condition of the deceased, and documentation of eventual traumatic lesions were analyzed. Discrepancies between gross anatomic and microscopic findings were also studied.

**Results:** The mean age of the population was 46.2 years (range 5-91 years). The sex ratio (H/F) was equal to 2.46. Bodies showed respectively putrefaction in 92 cases, mummification in one case and diffuse carbonization in 15 cases. Concerning manner of death, the majority of the cases were natural deaths (n=130, including 63 cases of sudden death), followed by suicide (n=113), accident (n=104). Homicide and undetermined manner of death were respectively found in 40 and 41 cases. The most frequent causes of death were blunt force injuries (n=73), cardio-vascular diseases (n=90), mechanical asphyxia including drowning (n=62), acute intoxication (n=59) and gunshot wounds (n=47). No cause of death was found in 32 cases. Mechanism of death not shown by gross anatomic findings was discovered by histology in about 40% of the cases (n=173). The main mechanisms of death found were respectively cardiac arrhythmogenic substrate (n=98), acute myocardial ischaemia (n=17), pulmonary infection (n=17), vital alimentary aspiration (n=14), fat embolism (n=13), pulmonary thrombo embolism (n=5), diffuse axonal injury (n=3), disseminated intra-vascular coagulation (n=2) and sickle cell crisis (n=2). Cause of death was established only by histology in 8.4 % of the cases (n=36). In the 32 cases for which no cause of death was found, histology showed possible mechanism of death in 11 cases corresponding to a cardiac arrhythmogenic substrate. Microscopic findings affected the manner of death in 13% of the cases (n=56). Histology provided complementary information about prior medical condition of the deceased in about 49% of the cases (n=211). Traumatic lesions were better documented by histology in about 22% of the cases (n=94). The majority of discrepancies between microscopic and gross autopsy findings involved the liver, the heart, and the lungs. According to these results, microscopic findings are relevant if adequate sampling for histology is performed during autopsy. In most of the studied cases, histology can be considered contributory regarding respectively mechanism, cause and manner of death, prior medical condition of the deceased and traumatic lesions documentation.

**Conclusions:** According to the results of this study, systematic standard histology for the main organs should be used in routine forensic autopsies.

**Forensic Autopsy, Histology, Cause of Death**

# G112    Radiology Students and Morgues: A Mutually Beneficial Relationship

*Nancy S. Adams, BS\*, 202 Milford Street, #155, Tupelo, MS 38801*

After attending this presentation, attendees will be better informed of the benefits that may be realized by affiliating with a radiologic technology program to provide radiology students with clinical morgue experience.

This presentation will impact the forensic community by making people aware of the improvements to radiographic image quality that may be accomplished when radiology students are allowed to observe autopsies and assist with forensic radiographs. A secondary benefit is recognized in preparing the radiologic technologist to assist with other skills such as evidence collection and preservation.

Interest in the forensic sciences has grown significantly in recent years as events and the media have focused attention on forensic investigations, and the radiologic sciences are no exception. Due to most morgues and medical examiner facilities being totally separate from hospitals today, the radiology student and radiology practitioner do not have adequate experience in forensic imaging. As radiology equipment becomes more and more sophisticated, and imaging techniques such as virtual autopsies and 3D CT reconstruction are utilized more frequently, the skills of the board-certified technologist will be in greater demand. Due to the nature of the work, it is important for the student to have some knowledge of the expectations and working conditions to aid in determining if this is a field they may wish to pursue. Just as the student obtains knowledge and experience in a broad range of imaging modalities to decide on a career path after graduation, an introduction to the morgue and forensic imaging should be available as well. Many times the radiologic technologist must image living and deceased

subjects in the emergency department, and if that technologist has some forensic training and background, preservation of evidence would be complied with, observation and interview skills would be enhanced, and the images obtained would provide proper legal documentation.

The morgue or medical examiner's facility can benefit by having students and instructors available who are well-versed in recognizing imaging artifacts, equipment and image processing malfunctions, and are able to troubleshoot and correct or at least identify the problem. They can develop proper exposure techniques and set up guidelines; train morgue assistants in obtaining better images; recognize foreign objects and implants; position to overcome superimposition of structures or demonstrate an anatomical part more accurately. In addition, the instructors work with the students to compare ante-and postmortem images and reproduce an antemortem position if necessary for comparison. An additional benefit includes access to board-certified instructors who are available to the facility for consultation, physically or electronically. A financial benefit to the facility may also be considered, as the students and instructors are not paid employees, and may assist in reducing overhead by maintaining the x-ray and image processing equipment in proper working order. And as a final benefit, the facility may be able to recruit exceptional candidates for employment, many of whom will have a bachelor's degree and may seek additional training as a multi-skilled individual.

This presentation describes an arrangement between a radiologic technology program and a medical examiner's facility and the mutual benefits both groups have enjoyed to date, including the development of a forensic radiography handbook suitable for both the novice radiographer and the morgue assistant involved in taking forensic x-rays.
**Radiology, Student, Education**

## G113 CT Autopsy Imaging in the State Medical Examiner Setting: Logistic Issues, Techniques, and Findings

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The goals of this presentation are to describe this research experience with logistic and technical aspects of the development of a CT autopsy imaging service for the state medical examiner's (ME) investigation of traumatic death, describe and compare CT imaging autopsy appearances with the ME's autopsy findings, and consider the future potential of CT imaging autopsy.

The logistic and technical challenges to the development of a CT autopsy imaging service require educational efforts and infrastructure development. Imaging autopsy is an accurate tool for the detection of most major injuries and causes of death resulting from blunt trauma or drowning. This presentation will impact the forensic science community by demonstrating how CT imaging autopsy has the potential to replace conventional medical examiner autopsy in some deaths resulting from accidental blunt trauma and may facilitate rapid retrieval of ballistic fragments in cases where forensic autopsy is required.

The medical examiner community has shown interest in the use of CT autopsy imaging, but faces problems of access due to financial, technical, transportation, interpretation, and related difficulties in incorporating this tool into regular practice as compared to CT imaging

in the clinical care of the living. Sharing collective experience, both positive and negative, in addressing these issues is important in identifying its long-term role in the medical examiner setting.

The logistic and technical challenges to the development of a CT autopsy imaging service require educational efforts and infrastructure development. Imaging autopsy is an accurate tool for the detection of most major injuries and causes of death resulting from blunt trauma or drowning. CT imaging autopsy has the potential to replace conventional ME autopsy in some deaths resulting from accidental blunt trauma and may facilitate rapid retrieval of ballistic fragments in cases where forensic autopsy is required.
**CT Imaging, Autopsy, Logistics**

## G114 Intersecting Fractures of the Skull and Gunshot Wounds: Case Report and Literature Review

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After attending this presentation, attendees will have a clear example of Puppe's rule utility in gunshot wounds analysis and will learn the advantages of a Multi-Slice Computed Tomography approach in such cases.

This presentation will impact the forensic community by demonstrating the ability of CT scanning to show gunshot wounds to the skull vault including entrance wound, exit wound with beveling, direction of the bullet path as well as differentiation between entrance and exit wounds using intersecting fractures (Puppe's rule).

This paper highlights the ability of CT scanning to show gunshot wounds to the skull vault including entrance wound, exit wound with beveling, direction of the bullet path as well as differentiation between entrance and exit wounds using Puppe's rule.

This rule, established by the German forensic pathologist Puppe in 1903, states that when two or more fracture lines of the skull produced by different blunt forces intersect, it is possible to reconstruct the sequence of injuries.

The intact skull allows fracture lines to develop normally while the presence of bone damages causes the subsequent injuries to stop in the point of intersection with the previous wounds. In other words this means that fracture lines produced by subsequent impacts are arrested at pre-existing fractures of the skull.

No exceptions to this rule have been found in systematic investigations on skulls, glass, and eggs stricken with subsequent blows.

Although multiple gunshots cause an extensive and sometimes very complex pattern of fractures due to the hydrodynamic effect produced by the bullet traversing the temporal cavity of the brain, in the majority of cases, Puppe's rule can be usefully applied.

This principle gains interest in sequencing multiple gunshot injuries and in determining the direction of fire. However, it may be useful also in differentiating entrance from exit wounds, especially if specific distinguishing features are absent (i.e., internal/ external beveling of the skull).

Herein a case of a 76-year-old man who shot himself in the mouth with a Walther PPK 7.65 handgun (caliber 9x17 mm) is reported.

Prior to autopsy a total body multislice computed tomography scan (MSCT) was performed. MSCT scanning was executed on a Mx 8000

Quad Diamond select unit (Philips Medical Systems, Andover, MA). In areas of forensic importance, axial MSCT was performed with 4 x 1, 25 mm collimation. The duration of MSCT scanning was approximately 15 min. Using an open-source workstation (OsiriX version 3.1) it was possible to calculate two-dimensional sagittal and coronal reformations and three-dimensional reconstructions.

Major radiological findings were: a bone defect of the hard palate, a complex pattern of fractures of the ethmoid bone with hemorrhagic filling of the ethmoid sinus, an anterior pneumoencephalus, a fracture of the anterior cranial fossa and a bone defect of the vault with external beveling of the outer table.

The abrupt termination of a fracture line belonging to the exit wound pattern (parietal bone) at a pre-existing damage caused by the entering bullet (temporal fracture originating from the entrance wound), well documented by the 3D-CT reconstruction, was used as an adjunctive tool to better distinguish the entrance from the exit wound.

This paper describes a clear visual example of Puppe's rule utility in the analysis of gunshot injuries of the skull and highlights the importance of postmortem forensic radiology.

In fact, MSCT allowed the investigation of the anatomical sites that are hardly accessible at autopsy (such as paranasal sinuses, temporal and ethmoid bones, etc.) and offers different views and angles of imaging improving the quality of the investigation.

**Gunshot Wounds of the Skull, Intersecting Fractures, Puppe's Rule**

## G115 Sudden Cardiac Death Due to Atrophy and Fibrous and/or Fatty Substitution of Right Ventricle: Pathologic Substrates and Postmortem High Resolution MRI

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After attending this presentation, attendees will be introduced to some cases arrived to observation of Section of Legal Medicine of Palermo about sudden cardiac arrhythmogenic death, in order to show the possible disease that can cause death. This presentation emphasizes the difficulty of making diagnosis of Arrhythmogenic right ventricular cardiomyopathy (ARVC) without genetic analysis: myocardial atrophy and fatty/fibro-fatty substitution is diagnostic of ARVC at autopsy only in absence of any other cardiac and extracardiac remarkable injuries.

This presentation will impact the forensic community by demonstrating how data showed highly frequent association between ARVC and the fatty variant with cardiomyopatic pattern. MRI is more sensitive to detect the fatty variant with the caridomyopatic pattern rather than fibro-fatty and/or infiltrative substrate. With this presentation authors will show cases reached to their attention in the last years, characterized by atrophy and fibrous and/or fatty substitution of right ventricle's muscle, with consequent sudden cardiac arrhythmogenic death. Besides, authors will try to make differential diagnosis between different nosologic entities compatible with histological findings, in order to reach the most probable diagnostic hypothesis.

ARVC is a primitive disease of myocardium with not-well-known aetiology, characterized by structural and functional abnormalities of right ventricle, that consist in an hemodynamic, rhythmic and electric disarrayes, due to progressive myocardial atrophy with fatty or fibro-fatty substitution.

Sectorial or widespread atrophy of the myocardium of free wall of right ventricle is the typical morphologic case, with normal characteristics of left ventricle and interventricular sept. Loss of myocardium set the trans-lightning of the wall, that appears to be yellowish for the fatty substitution and/or whithish for the fibrous substitution. Another constant find is the right ventricular dilatation, from mild to severe, with hypokinesia or akinesia of the muscle.

Less frequent are noticed single or multiple right parietal aneurysms, more often at the back side, infundibulous or apex. Besides, frequently there's mural thrombosis of right ventricle and septal fibrosis. This can cause a consequent bilateral atrial dilatation with the high risk of auricular thrombosis with possible systemic and pulmonary embolism.

From 1990 to 2006 474 autopsies of sudden cardiac death (SCD) were performed. In this series, ARVC accounted for 27 cases (16.4%), including 21 males and 6 females (16 to 43 years old; median 26 years). Circumstances of death were during physical exercise in 20 cases, and at rest in seven cases. The family history of ARVC and/or SCD was negative.

The transmural loss of the myocardium in the right ventricular free wall was diffuse in 11 cases and segmental in 16 cases. External bulging of right ventricular free wall and left ventricular was present in four and six cases respectively. The myocardial atrophy was replaced by fatty tissue in 20 cases and by fibro-fatty tissue in seven cases. The interface between residual cardiomyocytes and tissue replacing the myocardium (fatty or fibro-fatty), was wave front (cardiomyopathic pattern) in 21 cases, and lacelike (infiltrative pattern) in six cases. Active myocarditis was detectable in the fibro-fatty variant only. MRI describe the fatty replacement on T1-weighted images with moderate inter-intra observer variability, thus sequende like "fat suppression" or "triple inversion recovery" to evaluate fatty replacement might provide a significant improvement in the diagnosis of ARVC.

The data showed highly frequent association between ARVC and the fatty variant with cardiomyopatic pattern. MRI is more sensitive to detect the fatty variant with the caridomyopathic pattern rather than fibro-fatty and/or infiltrative substrate.

With this presentation cases reached to their attention in the last years, characterized by atrophy and fibrous and/or fatty substitution of right ventricle's muscle, with consequent sudden cardiac arrhythmogenic death will be discussed. Besides, authors will try to make differential diagnosis between different nosologic entities compatible with histological findings, in order to reach the most probable diagnostic hypothesis.

**Sudden Cardiac Death, Arrhythmogenic Right Ventricular Cardiomyopathy, Magnetic Resonance Imaging**

## G116 Autopulse® Associated Injuries

*Kathryn H. Haden-Pinneri, MD\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054; Dwayne A. Wolf, MD, PhD, Harris County Medical Examiner's Office, JAJ Forensic Center, 1885 Old Spanish Trail, Houston, TX 77054; and Jennifer C. Love, PhD, and Roger A. Mitchell, Jr., MD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

By attending this presentation, attendees will become familiar with the Autopulse® resuscitation device and the variety of internal injuries that can be associated with its use.

This presentation will impact the forensic community by educating forensic pathologists about the visceral and skeletal injuries associated with the use of automated chest compression devices so that they are not misinterpreted as perimortem trauma.

Automated devices have been utilized to assist with cardiopulmonary resuscitation (CPR) for many years. The most commonly encountered device is an Automated External Defibrillator

(AED) which provides rapid defibrillation for those patients with shockable rhythms. The newest device is a portable automatic external chest compression device. The Autopulse® Non-Invasive Cardiac Support Pump is one of these devices. Originally developed by the Revivant Corporation and subsequently purchased by Zoll Medical Corporation, the Autopulse® provides consistent, uninterrupted chest compressions through a load distributing band which squeezes the entire chest. Utilizing the device frees the emergency medical technician normally assigned to do chest compressions to perform other life saving activities while transporting the patient.

This FDA-approved device consists of a short backboard and a load distributing disposable compression band, called a LifeBand®. The patient is placed on the board and the band is secured around his or her chest. The band automatically sizes the patient, calculating the size, shape and compliance needed without requiring any patient information to be entered. Once started, the Autopulse® rhythmically constricts the entire rib cage, compressing at a rate of approximately 80 beats per minute. The LifeBand® can be placed over AED pads, but must be removed for standard paddle defibrillators. Compressions continue for as long as necessary or until the device is stopped. Standard protocol is for patients to receive manual chest compressions before the device is started.

Houston, Texas became a test center for the use of the Autopulse® with devices installed in all Houston Fire Department first responder vehicles. During a two month period, the Autopulse® was utilized on 264 patients, 156 (59%) of whom died and met criteria for medical examiner jurisdiction. Of these cases, 54 (35%) were autopsied. Nearly all patients had the external stigmata associated with Autopulse® use, a finding previously reported in the literature. More importantly, though, a significant number had internal injuries. The most common finding, after the external abrasions, is posterior rib fractures associated with posterior intercostal muscle hemorrhage, an injury previously not associated with manual chest compressions. Other injuries include liver and spleen lacerations, hemoperitoneum, vertebral body fractures, and mesenteric lacerations.

A subsequent study at the Harris County Medical Examiner's Office of patients who were resuscitated utilizing the Autopulse® was undertaken to determine if body habitus or bone strength played a role in occurrence of injuries. A total of 58 cases were reviewed and 36 (62%) were found to have posterior rib fractures. Rib fractures occurred fairly equally amongst younger individuals with robust bone as well as older individuals with osteoporosis. Furthermore, the overall size of the chest did not appear to be associated with an increase or decrease in rib fractures or visceral injuries. However, band placement, evidenced by the characteristic skin abrasions, and body habitus were associated with bone and visceral injuries.

Data is still being analyzed in the utility of the Autopulse® in mainstream resuscitation. Some studies have shown improvement in survival over manual compressions, while others did not. Over 4300 units have been installed and approximately 24000 LifeBands™ have been used clinically, and these numbers are likely to increase. A comprehensive understanding of biomechanics and resultant bone and visceral injuries is crucial for forensic pathologists charged with the responsibility to differentiate between Autopulse® and traumatic injuries. It would be prudent to have a policy in place with the EMS responders in your area such that they are not used on patients with traumatic injuries and they should never be utilized on children.

**Autopulse®, Autopsy, Posterior Rib Fractures**

## G117 Migration of a Bullet in the Lumbosacral Spinal Canal: A Case Report and Review of the Literature

*Audrey Farrugia, MD\*, Bertrand Ludes, PhD, Annie Geraut, MD, and Jean Sebastien Raul, PhD, Medicolegal Institute, 11 Rue Humann, Strasbourg, 67065, FRANCE*

After attending this presentation, attendees will understand rare cases in common medicolegal autopsy practice, descriptions of different types of spinal canal migration reported in the literature, and mechanisms and conditions that could induce a bullet migration.

This presentation will impact the forensic community by explaining a well known but relatively rare phenomenon in common medicolegal autopsy practice.

Reported here is a case of a 22-year-old man who was hit by a gunshot on the left part of the chest in the sixth intercostal space. The bullet had a previous course in a first victim.

The X-Ray examination of the body of the 22-year-old man showed a metallic object projected at the level of L1. At autopsy, examiners described that the bullet during its trajectory induced a pulmonary lesion, gastric perforation, pancreas, and hepatic lesion and left renal vein and artery perforation. The bullet then entered the spinal canal through the L1-L2 intervertebral foramen. Classical laminectomy at the lumbar level was performed, the dura was opened, and the bullet was found at the L5-S1 level. This case illustrates a bullet migration due to gravitational forces as its kinetic energy decreased after an intermediary target (the first victim) and collision with body structures.

The different cases reported in the literature, particularly the different types of migration will be discussed. If migration of T10-S1 seems to be more frequent, some authors reported a C1 to S2 or cranium to L4 migration. In one case, a migration from S1 to L4 level in the cranial direction, because of the patient's position, was also described. The different mechanism of migration and the symptomatic effect of this phenomenon will also be discussed.

**Bullet, Migration, Spinal Canal**

## G118 Homicide-Suicide in Tours France: 2000-2007

*Pauline Saint-Martin, MD\*, and Patrick O. Byrne, MD, Service de Medecine Legale, Hopital Trousseau, Centre Hospitalier Régional Universitaire de Tours, Tours, 37000, FRANCE*

After attending this presentation, attendees will learn about the characteristics of homicide-suicide in a French rural area in an eight-year period, as only one study on these events has been conducted in France.

This presentation will impact the forensic science community by expanding knowledge of homicide-suicide in France. An important stage could be the creation of a national surveillance network, so that studies could be conducted over the whole country and over a long time span, allowing the possibility of preventive intervention.

**Introduction**: Homicide-suicide (HS) has been defined by a homicide committed by a person who subsequently commits suicide within one week of the homicide. These events are relatively uncommon but they have often drawn the media's attention. HS occupies a distinct epidemiological domain that overlaps with suicide and domestic homicide. Two major classifications have been proposed. Both are based on victim-offender relationship and motive. Few statewide studies of HS events have been conducted, but no data from France are available, except one Parisian study. This study of the characteristics of

all HS in Tours, France during an eight-year period was compared with results obtained in other international studies.

**Material and Methods**: Case records of the Institute of Forensic Science of Tours were reviewed for 2000-2007. The age and sex of the perpetrator and victim, the relationship between them, the method of death, and the circumstances were noted.

**Results**: Sixteen HS involving a total of 33 decedents occurred during the 8-year period. It represents 11% of the total case of homicides within this period, for a population of 871,000 persons. All offenders were male, with a mean age of 68 years. In 11 events (68%), the offender used a rifle for both the homicide and suicide. Most victims were female (14/17), with a mean age of 64 years. Fourteen events occurred at home. Five were suicide pacts, during which one person killed the other before committing suicide. In these cases, a suicide note was always found.

**Discussion**: Data were similar to those obtained in other studies. The victim was often a female who was younger than the offender and her intimate partner. The most frequent apparent motive was the breakdown of the relationship. Suicide pacts were also frequent. Shooting was the most frequent used method of both homicide and suicide. Availability of firearms in this rural area of France can be explained by an important activity of hunting. The percentage of HS related to the total number of homicides was important and this result confirmed one epidemiological law of HS: the lower the homicide rate, the higher the percentage of HS. One limitation of study was the limited number of cases. However, a research strategy should be developed in France to expand knowledge of these events. A phase of this strategy could be the creation of a national surveillance network, as well as preventive interventions.

**Homicide-Suicide, Suicide Pact, Amorous Jealousy**

## G119 Sudden Deaths Associated With Sexual Activity

*Albert Y. Chu, MD\*, Sharon M. Derrick, PhD, and Luis A. Sanchez, MD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

Attendees will review of non-homicidal sudden deaths associated with sexual activity that occurred in Harris County, Texas, from January 2004 until the present. The goal of this presentation is to describe the epidemiological characteristics, autopsy findings, and toxicology results in this group of decedents.

This presentation will impact the forensic community by describing a population of decedents that commonly present to medical examiner and coroner's offices, including a description of the relatively high prevalence of substance abuse in this population.

Because deaths associated with sexual activity often occur suddenly and outside of the care of a physician, they are frequently reported to the local medical examiner or coroner's office. The extent of the resulting death investigation may range from no additional investigation to a full autopsy with histologic and toxicologic evaluation, depending on the circumstances of the particular case, office-specific guidelines, and the judgment of the individual pathologist/investigator. To further characterize this group of decedents and thereby aid in the investigation of similar deaths in the future, a review of sudden deaths associated with sexual activity was performed.

A review of cases from the Harris County Medical Examiner's Office from January 2004 until the present identified 35 cases of sudden death associated with sexual activity (excluding homicides). These cases fell into three broad categories: (1) collapse occurring either during or immediately around the time of sexual intercourse (22, 62.9 percent), (2) individuals found unresponsive in adult-oriented establishments (theaters, video booths, etc) (11, 31.4 percent), and (3) cases of autoerotic asphyxiation (2, 5.7%).

Autopsies were performed in 29 of 35 (89.7%) of all cases and toxicology testing (including at least a screen for stimulants) was performed in 25 of 35 (71.4%) of all cases. The population was overwhelmingly male, with a male to female ratio of 10.7 to 1. The mean age was 57.6 years. Atherosclerotic and/or hypertensive cardiovascular disease represented the most common natural cause of death, and was identified in 29 of 35 (82.9%) of all cases. The mean heart weight was 543.9 grams among natural deaths and 490.0 grams among deaths attributed to substance abuse.

Deaths were attributed to substance abuse in nine of 35 (25.7%) of all cases and nine of 25 (36.0%) of cases in which toxicology testing was performed. Of these, only five of nine (55.6%) had a known history of substance abuse. Among cases in which toxicology testing was performed, substance abuse-related deaths occurred in five of 14 (35.7%) of individuals having sexual intercourse and four of nine (44.4%) of individuals found in adult-oriented establishments. Cocaine was the most commonly identified drug of abuse, followed by methamphetamine and methylenedioxymethamphetamine. One case of inhalant abuse (ethyl chloride) was identified. Atherosclerotic and/or hypertensive cardiovascular disease was identified in six of nine (66.7%) of deaths attributed to substance abuse.

The high prevalence of cardiovascular disease that researchers observed is consistent with previous studies of myocardial infarction and/or sudden death associated with sexual activity. Less recognized by the existing medical literature is the significant prevalence of substance abuse in this population, including those decedents with no known history of drug use. The results of this review suggest that a history of sudden death in association with sexual activity warrants at least a toxicologic analysis for stimulants in order to classify accurately the cause and manner of death, even in the absence of a known history of substance abuse.

**Sudden death, Sexual, Toxicology**

## G120 Sex Killer: Sexually Related Trauma and Deaths - Forensic Aspects

*Gilles Tournel, PhD\*, IML de Lille, 1, Place de Verdun, Faculté de Médecine, Lille, 59045, FRANCE; and Valery Hedouin, PhD, Cédric Houssaye, MD, Frédéric Grimopont, Antoine Stelandre, Anne Becart, PhD, Viviane Gnemmi, Sandrine Depret, MD, IML de Lille, Lille, 59045, FRANCE; and Didier Gosset, PhD, IML de Lille, Faculte de Medecine, Lille, 59045, FRANCE*

After attending this presentation, attendees will know the description of potentially dangerous sexual practices and their consequences involving death.

This presentation will impact the forensic science community by the knowledge and the awareness of these particular sexual practices and their consequences involving death.

**Introduction:** Many men and women practice a broad range of voluntary sexual activities, most of which are harmless. Many minor injuries of the genital, oral, and anal areas do occur but most of them only require symptomatic therapy. The only erotic activities with an unacceptable risk for injury are vaginal insufflations during pregnancy, and fist fornication. Some forensic deaths are indirectly linked to sexual activity. Three cases of deaths with unusual fatal mechanisms during sexual activities are reported.

**Materials and Methods:** Forensic investigations of the crime scene and the autopsy findings of three cases: two women and one man will be presented. Another living woman with important genital lesions provoked by sexual activity was examined by a forensic pathologist and gynecologic doctor. The pelvic and abdominal lesions are described.

**Results:** In Case 1, a 35-year-old woman was found dead in her bed. The death was attributed to asphyxia by strangulation. At autopsy,

anal and sphincter injuries and massive rectal hemorrhage were seen, due to rectal fist insertion (fisting). Her husband was sentenced for murder by strangulation and sexual assault. Case 2 presents a 42-year-old man was found dead in his car in the driver's seat, his clothes (trousers and underpants) pulled down around his ankles. Police and forensic investigations supposed voluntary sexual practices with a prostitute, such as oral sex. At autopsy, investigators observed an internal thoracic hemorrhage linked to a ruptured aortic aneurysm. The sexual activity brought about the tearing of aortic tissue. Case 3 presents a 48-year-old woman performed voluntary sexual activities such as vaginal and rectal fist and foreign body insertion (alcohol bottle into the base) during heterosexual activity. The surgeons observed haematomas of the vulva, major labia, minor labia, and anal area. A colostomy was performed for the anal sphincter injuries and a surgical act to drain off the haematomas. In Case 4, a 37-year-old woman was found dead in her bed. A large quantity of blood was observed between and over the thighs. Her husband specified that the blood resulted from menstruation. At autopsy, vaginal injuries and massive hemorrhage were observed, due to vaginal fist insertion (fisting). The cause of death was vaginal hemorrhage due to the fisting. Toxicological analysis showed she was drunk. Her husband pretended that she consented to this sexual activity. He was sentenced for sexual assault leading to the death by hemorrhage.

**Discussion:** Anal and/or vaginal fist or foreign bodies being inserted are uncommon and potentially dangerous sexual practices. Forensic investigations, the autopsy, toxicological and histopathological findings, and the manner and the mechanism of death for three persons will be discussed. The insertion of a clenched hand and forearm into the vagina or rectum during heterosexual activity and indirect performing aortic rupture on pre-existing lesions during oral sex are linked to the cause of death. The frequency of such fatal outcomes or sexual activity of anal and vaginal penetration, the injuries observed, the cause of death due to theses acts (exsanguinations by traumatic damage to the canal anal and to the vagina or/and air embolism), the consequences of these practices, the relationship between the perpetrator and the victim and the special features at the scene, are discussed and compared to the literature. Indeed, foreign bodies, arm, and forearm inserted into the rectum and the vagina with associated hemorrhage and perforation have been well documented in medical literature. However, death following these acts has very rarely been reported. Such cases remain rare but have to be reported to alert the forensic pathologists, investigators, and coroners. In a larger range, the public must be aware of the role of such sexual activity and their consequences involving death.

**Fisting, Hemorrhage, Erotic Death**

# G121 Conducted Electrical Weapons — A Review of the Medical Literature

*Donald M. Dawes, MD\*, Lompoc Valley Medical Center, 518 East Hickory Avevue Lompoc, CA 93436; and Jeffrey D. Ho, MD\*, Hennepin County Medical Center, 701 Park Avenue South, Department of Emergency Medicine, Minneapolis, MN 55415*

After attending this presentation, attendees will have gained a working knowledge of the existing medical literature on conducted electrical weapons such as the TASER X26.

The presentation will impact the forensics community by improving knowledge of the existing medical research on conducted electrical weapons which may be important especially for those who make determination of death decisions.

Conducted electrical weapons are used to control violently resistive subjects. The devices discharge a small electric current that stimulates both afferent sensory neurons causing pain and efferent motor neurons causing involuntary sub-tetanic muscle contraction. The use of these devices is growing in the United States since it fills a large void in the use-of-force continuum. There is controversy in the lay press and medical literature regarding the use of these weapons and the sudden, in-custody death phenomenon. Some groups have claimed that these devices have caused several hundreds of deaths. This presentation is a review of the existing medical literature on these devices. By attending this presentation, attendees will develop a comprehensive understanding of the existing medical literature on conducted electrical weapons and will develop and understanding of questions that remain to be answered. The existing animal studies, case reports, and human prospective studies will be examined. The focus will be particularly on the existing prospective human research.

The presentation will examine issues such as cardiac safety, respiratory effects, and the impact of the devices on other physiologic parameters such as blood chemistries, pH, stress markers, and temperature. The presentation will examine the use of these devices in the presence of cocaine, and in the presence of cardiac pacemakers and internal defibrillators. The difference between animal data and human data will be discussed.

It is important for the forensic community to be knowledgeable about the existing research and the questions that are still not answered, particularly those individuals who make determinations of cause of death.

**Conducted Electrical Weapon, TASER, In-Custody Death**

## H1    Assessment of Differences in Decomposition Rates of Rabbit Carcasses With and Without Insect Access Prior to Burial

*Jutta Bachmann, MSc\*, Postweg 2, Fellbach, Baden-Wuerttemberg 70736, GERMANY*

After attending this presentation, attendees will have an understanding of differences in decomposition of buried remains with and without insect access prior to burial. This will assist in forensic cases where victims are frequently buried to remove traces of evidence, to conceal the crime itself, and to delay discovery and identification.

This presentation will impact the forensic community by providing regression formula for buried remains with insect and without insect access to allow a more precise estimation of the PMI. It will underline the fundamental importance of insect impact on decomposition rate and pattern.

Although progress in estimating PMI for surface remains has been made (Megyesi, et al. 2005), no previous studies allow this to be accomplished for buried remains.

Limited research has been conducted to assess decomposition pattern of buried remains, or the impact of insect access on burial decomposition in relation to Accumulated Degree Days (ADD). Considering the well-known importance of insects on the progress of surface decomposition, it was expected that pre-burial insect access to a carcass would equally result in enhanced decomposition rates.

A burial study on 60 rabbit carcasses was carried out in northwest England to assess differences in decomposition rates in carcasses with insect access prior to burial, and those without. Individually buried cadavers in 35 cm soil depth were exhumed at 50 ADD intervals, and Total Body Score (TBS), weight loss, carcass/soil interface temperature, and underneath carcass soil pH were assessed.

A comparison between decomposition rates between the two groups in relation to log ADD indicated a highly significant faster decomposition rate ($p \leq 0.001$) in the Insect group than the Non-Insect group. The following regression formulae were developed by statistical Analysis of Co-variance (ANCOVA):

Insect group: TBS = -39.4+23.38 x log ADD
Non-insect group: TBS = -29.49+17.63 x log ADD
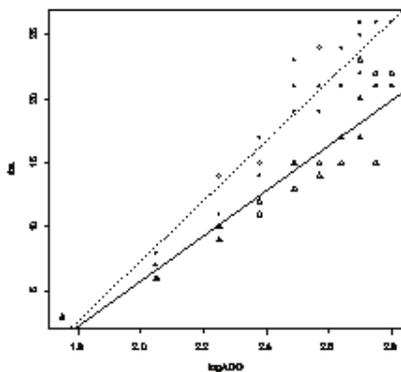


**Figure 1:** Regression lines of TBS against log ADD of Insect (indicated by circles) and Non-Insect group (indicated by triangles), showing enhanced decomposition in the Insect access group.

Preferred initial oviposition sites of blowflies in the genital area coincided with further localized decomposition progress, skeletonization, and disarticulation. Basic decomposition patterns in both groups were similar, but first appearance of hallmarks of the various decomposition stages in the Insect group preceded their appearance in the Non-Insect group by 200 ADD. At a total study interval of 641.87 ADD this represented an approximately 30% enhanced decomposition in the carcasses with insect access prior to burial. Thus, the effectiveness of TBS in precisely reflecting decomposition stages in relation to ADD is confirmed.

Intra-abdominal liquefaction occurred during advanced decomposition stages, exhibiting an ADD-related pattern. Parallel to external decomposition characteristics, the Insect group preceded the Non-Insect group by approximately 200 ADD. There is potential to refine the TBS scoring system by incorporating features of internal decomposition.

True larval masses were never observed during the course of this study, thus interface temperatures did not exhibit significant differences from reference soil temperatures. Correspondingly, there were no significant differences between interface temperatures in the Insect and Non-Insect groups.

Weight loss between the two groups presented significant differences of low magnitude, but was subject to measurement bias due to soil adherence. Soil pH was shown to peak at 311.12 ADD, parallel to increased weight loss, but did not closely reflect earlier or later decomposition rates or patterns.

Insect stages were collected and reared to adulthood and included mainly the order Diptera, family Calliphoridae (*C. vomitoria spp., C. vicina spp.*); there was sporadic presence of few insects of the order Coleoptera, family Carabidae.

The results of this study must be further tested, as thus far there is paucity of data on ADD in decomposition, which does not allow useful comparisons between studies.

**Burial, Decomposition, Insects**

## H2    Bugs Bunny?  No Bugs Bunny

*Tal Simmons, PhD\*, Peter A. Cross, MSc, Rachel Adlam, MSc, and Colin Moffatt, PhD, University of Central Lancashire, School of Forensic and Investigative Sciences, Preston, Lancashire PR1 2HE, UNITED KINGDOM*

After attending this presentation, attendees will gain an understanding of the impact of insects on the decomposition process in relation to accumulated degree days (ADD) regardless of the environment in which the carcass is deposited.

This presentation will impact the forensic community by influencing the way in which postmortem interval (PMI) estimates are constructed. The data presented show that, when time/temperature is standardized as ADD, it is the presence of insects alone that accelerates decomposition. This holds true for both surface-deposited remains and for buried remains. Burial is a factor in decomposition solely because it normally excludes (or reduces, depending on the duration of the exposure interval between death and burial) oviposition by Diptera species. ADD can be used to estimate the PMI in burials (using an insect exclusion model) in the same manner as it is in surface depositions.

When temperature/time is standardized as Accumulated Degree Days (ADD), insect access is the most influential factor affecting

decomposition rate. Carcass size is of secondary importance, with smaller carcasses decomposing faster than larger ones, mainly due to insect clutch size, which affects larval numbers and the amount of heat generated by the larval mass, which in turn accelerates insect developmental trajectory. Carcass disturbance is also a factor affecting decomposition, particularly when measured via percent weight lost, as movement of a carcass disrupts the insect feeding activity and delays skeletalization.

This paper explores the effect of insect exclusion on decomposition rate in a direct comparison of insect accessed and insect excluded carcasses of the same size class, using a wild rabbit (*Oryctolagus cuniculus*) model. All rabbits were killed as part of the annual cull and their use was approved by university animal research ethics committees and official veterinarians of the United Kingdom Meat Hygiene Service. The studies were conducted primarily in the Northwest of England during the months May to July of 2008. Comparative data from Adlam and Simmons (2007) was used for insect exposed surface decomposition.

Insect accessed surface (N=24) and buried (N=30) carcasses as well as insect excluded surface (N=30) and buried (N=30) carcasses form the study set (total N=114). Insect excluded rabbits were placed in plastic bags immediately following culling, thus precluding oviposition. Buried rabbits were placed into graves immediately following weight recording and thermocouples were placed under the trunk of each rabbit to record changes in carcass temperature during underground decomposition. Two thermocouples were used to record ground temperature external to the graves. The burial depth for all rabbits was 30-35 cm, thus mimicking a shallow, clandestine grave. Similar protocols were followed with regard to the surface insect excluded rabbits, which were placed into raised cages screened with 1mm aluminum mesh and thermocouples were inserted subcutaneously in the abdomens of 7 randomly distributed carcasses. A thermocouple and data logger were also used to continuously record ambient temperature at the site. Carcasses becoming visibly infested with larvae were discarded from the experiment with no further data collected and appropriate control measures were taken to prevent further spread. For the buried, insect exposed carcasses, carcasses were left on the ground surface near the graves and exposed to normal insect activity for approximately five hours prior to burial. Insect exposed surface rabbits were laid on the surface under chicken wire fencing to prevent scavenging for the duration of the experiment.

The data collection protocol for both the buried and surface rabbits was carried out approximately every 50 ADD. This included weighing the rabbits, assigning a Total Body Score (TBS); and, for all groups except insect excluded surface depositions, taking soil samples for pH measurement.

The results indicate that oviposition occurred successfully on both insect exposed groups and was prevented (or delayed substantially) in the insect excluded groups. The rate of decomposition as measured by TBS in insect exposed carcasses exceeded that of insect excluded carcasses throughout the decomposition process with no overlap between the two groups. Furthermore, any difference in rate of decomposition between insect excluded groups (buried and surface) was not significant; the slopes of the regression lines (Figure 1) are not significantly different (p≤0.485). Buried insect-access carcasses displayed an intermediate rate of decomposition. This can be explained by the single episode of oviposition on the remains prior to burial, without subsequent insect infestation that would have further accelerated the decomposition process.
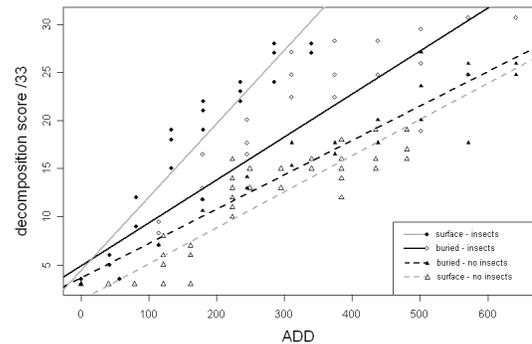


Figure 1: TBS vs. ADD for insect access and insect excluded rabbits

**Accumulated Degree Days, Burial, Insects**

# H3 Decomposition of Sharpey's Fibers in Estimating Postmortem Interval

*Gretchen R. Dabbs, MA\*, University of Arkansas, 330 Old Main, Fayetteville, AR 72701; and Michelle A. Granrud, 585 North Scottsdale Drive, Apartment 6, Fayetteville, AR 72701*

After attending this presentation, attendees will understand the potential for decomposition rates of Sharpey's Fibers of the alveolar bone to be used in estimation of Postmortem Interval using Accumulated Degree Days.

This presentation will impact the forensic community by introducing a new way to establish the postmortem interval after the shorter-term processes of algor, livor, and rigor mortis have completed, and in the absence of other common estimation methods such as forensic entomology.

After initial biological changes associated with *rigor*, *algor,* and *livor mortis* have run their course, the estimation of time since death becomes increasingly more difficult and less precise. Forensic entomology can extend the precision of postmortem interval estimation, but suffers when insect activity is non-existent and when time since death becomes increasingly long. Studies of decomposition rates of the soft tissue can add information to the estimation of postmortem interval, but to date, are limited in climatic region, thus making them less reliable in areas with different climates than the original study area. The utilization of accumulated degree-days for decomposition studies is useful, but often overlooked and marginalized. The single-rooted anterior dentition, particularly the incisors of both the maxilla and mandible, has been observed to fall out of socket after some period of decomposition. It is generally observed that this occurs after skeletonization, but it is unknown how long after. It is also unknown whether this is a regular process or a chance occurrence based on factors intrinsic to each situation. This paper reports the results of a series of experiments designed to test the number of accumulated degree-days necessary for the breakdown of the dental Sharpey's fibers, which serve to bind the cementum of the tooth roots to the alveolar bone. Pig (*Sus scrofa domestica*) dentition was utilized as a substitute for human dentition, as the facilities available are not suitable for human cadaver experiments. The study is limited to observation of the central maxillary and mandibular incisors, as these teeth have morphology quite similar to that of human incisors. None of the other dentition was observed for this study, but the entire dental arcade was exposed to avoid damage to the fibers in question during specimen preparation. To date, a total of seven experiments have been conducted; and more are planned for the upcoming months. Pig head specimens were collected from local butchers. Several were entire specimens with hide and bristles included, while others had been bisected along the mid-sagittal line and the hide

removed. Initial tests show no difference in Sharpey's Fibers decomposition rates between the two initial preparations. The test subjects were placed in a rural outdoor environment with mixed exposure to sun and shade. Large wire dog kennels which are open to the elements protect the test specimens. Two locations have been used to date, one being on the margin of a wooded area, the other in a more open pasture area. Each site was established with a DS1921G Thermochron from iButtonLink, which recorded outdoor temperature at hourly intervals from the initial positioning. Test specimens were checked on a daily basis for the first two weeks, and every three days subsequently. During the course of the check, each test subject was examined to identify whether the anterior teeth were still in place. The checks did not involve any contact with the test subject. Research to date suggests that at Sharpey's Fibers may decompose after only 3000°F accumulated degrees. Initial data shows decomposition of the alveolar Sharpey's Fibers sufficient for the loss of anterior dentition can occur in as few as 40 days of exposure during June and July (daily temperature average of 75.55°F). Further tests are currently being planned to support this data and identify which environmental conditions, besides temperature, have effects on the decomposition rates of alveolar Sharpey's Fibers. It is expected that this research will be useful in making more precise estimations of postmortem interval when that period is longer than can be measured using currently available methods.

**Forensic Anthropology, Postmortem Interval, Decomposition**

# H4 Year-of-Death Determination Based Upon the Measurement of Atomic Bomb-Derived Radiocarbon in Human Soft Tissues

*Gregory W. Hodgins, PhD\*, University of Arizona, Department of Physics, 1118 East Fourth Street, Tucson, AZ 85721*

After attending this presentation, attendees will understand how above-ground nuclear testing in the 1950s and early 1960s dramatically increased environmental levels of radiocarbon. These elevated levels have been incorporated into all organisms living since that time and thus can serve as temporal markers. Potentially, radiocarbon measurements of postmortem human tissues can be used forensically to establish Year-of-Birth and/or Year-of-Death. One advantage of this approach is that it functions independently of chemical or biological methods for the determination of Postmortem Interval, or Age-at-Death, and thus might augment current methods for establishing these parameters.

This presentation will impact the forensic community by providing data to assess the method's ability to predict Year-of-Death based upon soft tissue radiocarbon levels. The study quantifies precision and accuracy when applied to soft tissues from 36 known-age/known year-of-death human remains.

Environmental levels of radiocarbon spiked upwards from 1955 to 1963 due to above-ground nuclear testing and have been descending back towards natural levels ever since. It enters human tissues through diet; and growth and normal metabolic tissue replacement leads to the continuous incorporation of radiocarbon throughout life. Replacement rates vary between different tissues and so radiocarbon is unevenly distributed within the various tissue compartments of late 20th/early 21st century humans. Moreover, the distribution of radiocarbon in a specific individual is related to when he or she lived and died relative to the atmospheric radiocarbon spike and so the phenomenon has potential forensic applications. This particular study hypothesized that tissues with high replacement rates should have radiocarbon levels closest to the contemporary environment. Therefore, postmortem measurement of radiocarbon levels within such tissues should mark the Year-of-Death.

Radiocarbon levels were determined in blood, hair, nails, skin collagen, skin lipid, and bone lipid obtained from 36 individuals deceased in 2006. Levels were found to be lowest in blood, hair and nail samples and progressively increased in skin lipid, bone lipid, and skin collagen. Variation in radiocarbon levels within a tissue type was found to be surprisingly low, perhaps reflecting similarities in the diet of the sample population. Significantly, the magnitude of variation within the tissue populations was similar to the magnitude of the variation found in direct atmospheric radiocarbon measurements. Together, these observations allowed estimation of the method's precision. The best case measurement precision based upon fingernail radiocarbon measurements was a two sigma uncertainty of ± 2.5 years. However, precision varied considerably between tissues and is sensitive to death year. The accuracy of the method could not be quantified due to limitations of the published atmospheric radiocarbon data sets, however this is relatively straightforward to overcome.

This study is the most comprehensive empirical investigation of Year-of-Death estimation based upon tissue radiocarbon measurements to date. The method shows promise, however a broader study is necessary. Measurements of carbon and nitrogen stable isotopes in bone collagen purified from the study population indicated a surprising homogeneity in diet. This fact might have generated a spurious overestimation of the method's precision. Certain diets can alter tissue radiocarbon levels so it must be determined whether the observed dietary homogeneity was a quirk of the sample population or if it reflects widely shared behaviors. A second caveat is that published data on atmospheric radiocarbon levels extends only to 2003. Thus the present study estimated contemporary levels by extrapolation. Clearly the way forward is to expand the cultural and geographic diversity of test population and extend the reference data sets of atmospheric radiocarbon measurements up to the present.

**Radiocarbon, Year-of-Death, Human Tissues**

# H5 The Effects of Coverings on the Rate of Human Decomposition

*Angela M. Dautartas, BS\*, University of Tennessee, 250 South Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will understand some of the principles of human decomposition; specifically how decomposition can be affected by the presence of different materials surrounding a body.

This presentation will impact the forensic community by helping to expand the knowledge base of factors that influence the rate of human decomposition. This additional information will in turn aid investigators in more accurately predicting time since death in forensic settings.

A multitude of factors can affect each stage of the decomposition process, either accelerating the process or slowing it down, depending on the specific agent at work. Some of the most frequently observed variables are temperature, moisture, insect activity, and sun or shade exposure.

Coverings can impact several of these factors in the decomposition process, and are found frequently in forensic cases. In a survey of New Mexico cases, Komar[2] reported that sixteen individuals were found wrapped in plastic; and twenty were noted as wrapped in a cloth or blanket.

Variation in body coverings spans a wide spectrum. A case from Singapore involved the remains of a child found wrapped in nine layers of plastic and then placed in a plastic bag.[1] In this instance, the body was reportedly in a state of much higher preservation than expected for the climate;[1] illustrating how coverings can affect estimation of postmortem interval. In another survey conducted of eighty-seven cases, fifty-four of the bodies were wrapped in some covering prior to burial. Plastic was the most common, but a variety was noted, including rugs, sleeping bags, blankets, and clothing.[3]

In order to document how coverings could affect the decomposition process throughout the sequence, an experiment was designed to mimic a covered body in a forensic setting. Three human cadavers were used

in each repetition of this experiment. Two of the cadavers were covered, one in a plastic tarp, the other in a cotton blanket, while the third was left uncovered as a control. The selection of materials was based on case reports of cadavers wrapped in plastic and blankets (Komar, 2003, Derrick, 2007 personal communication). The cadavers were placed at the same time and in close proximity to ensure that all were exposed to similar environmental conditions. The cadavers used were also of the same sex and ancestry, and the age range and body weight variation was kept to a minimum to avoid extraneous influences on the decomposition process. All demographic information was recorded.

Data collected included daily minimum and maximum temperatures and two daily temperature point comparisons. The maximum and minimum temperatures allowed for calculation of accumulated degree days. The bodies remained covered and undisturbed for thirty days. At the end of that period, the bodies were uncovered, the amount of decomposition was recorded and the presence or absence of insect activity was noted.

Using the recorded temperature data, the accumulated degree days (ADD) was calculated and compared to the actual number of days postmortem. This technique provided a standard basis of comparison between the temperature data recorded from each individual and how temperature differences affected decomposition, particularly in the earliest stages.

Significant differences in temperature were found between the covered bodies and the uncovered cadavers through the use of paired t-test analyses. This indicates that the presence of a covering on a body will have a noticeable effect on the rate of decomposition. Differences were also identified in moisture content between the various shroud and surface environments. Variation between the calculated accumulated degree days and the actual number of days postmortem was less significant, but still showed marked differences. This again suggests that special consideration should be taken when estimating time since death in cases involving covered bodies.

**References:**
1. Chui PPS. 2006. An unusual postmortem change in a child homicide-leaching. Proc. American Association of Forensic Sciences. *12:*247 (Abstract).
2. Komar DA. 2003. Twenty-seven years of forensic anthropology casework in New Mexico. J Forensic Sci 48: 1-3.
3. Manhein MH. 1997. Decomposition rates of deliberate burials: a case study of preservation. In Haglund WD, Sorg, M. (eds). Forensic Taphonomy: The Postmortem Fate of Human Remains. New York: CRC Press, 469-482.

**Decomposition, Time Since Death, Coverings**

## H6 Modes of Mutilation in Taphonomic Context: Can Sharp Force Trauma Decelerate the Decomposition Process?

*Branka Franicevic, MSc\*, Department of Archaeology, Sheffield University, Sheffield, S1 4ET, UNITED KINGDOM*

The goal of this presentation is provide attendees information on the impact of mutilation practices on decomposition rates during the summer period in a terrestrial environment in a Mediterranean coastal region. The study will address aspects of dismemberment types that may delay decay rates, a process that is generally uncharacteristic for sharp force trauma.

Given favorable taphonomic conditions, body tissue incisions by sharp objects can significantly accelerate decomposition by attracting higher insect and scavenger succession, and by aiding more rapid internal and external bacterial action. It is however uncertain whether severing the limbs can affect decay rates in the reverse manner. Because the absence of the taphonomic model may pose a challenge to the estimation of postmortem interval (PMI) of mutilated remains discovered in advanced stages of decomposition, the consequent answers will impact on the forensic community and humanity by addressing the utility of taphonomic profiling in reconstructing postmortem histories. By providing a more coherent understanding of the decomposition processes involved, this study will consequently impact the forensic community by providing more precise estimations of PMI for the most frequent types of dismemberment.

The scientific justification of the research is based on the hypothesis that a different taphonomic model for mutilated body parts is possible, due to putrefaction being altered in the absence of the necessary gastro-intestinal organs responsible for bacterial activity. The alteration of intrinsic factors through dismemberment may cause more dependency on external factors in decomposition, consequently affecting rates of decay. This is because microscopic decomposers that are introduced from both the intestine and the outer environment are reduced mostly to the latter in the case of single-limb decay.

This experimental study involved a decomposition analysis of mutilated body parts in the terrestrial habitat in summertime on the south Croatian coast (43.02° N 17.57° E). It was conducted in June 2008 over a period of 30 days. Samples were completely exposed to the sun, deposited on salt marsh soil. Domestic pigs (*Sus scrofa*) were utilized as animal analogs to mimic human decomposition. The sample size was twelve animals: three whole carcasses (S1) were compared to three decapitated samples (S2), three animals with all limbs and the head severed (S3), and three samples mutilated in transverse plane (S4). The carcasses ranged in sizes from 21 to 23 kg., and they were kept in metal cages for the duration of the experiment to protect them from large scavengers. Temperature data were obtained from the local Hydro-meteorological Station. Once a day, micro-organisms were extracted and insects collected from the "drip zone."

The results demonstrated differences in the decomposition pattern between whole carcasses and within the three types of dismemberment. While all samples reached the decay stage, the bloating stage was omitted by S3 and S4. Significant differences in decay rates were noticed, with S3 reaching the decay stage within the first week followed by S4, S2, and S1 decomposing at the slowest rate. Entomological analysis indicated the highest succession on S3 with the intestines and the head being the preferred body parts in all three mutilated samples, followed by the body-tissue incision area. Most taxa were of the *Diptera* Order, belonging to seven Families and twelve species. Based on the microbial analysis, the species associated with decomposition on the mutilated remains was higher in number and more diverse than with whole corpses, with the highest fungal succession (*Ascomyceteous fungion*; *Aspergillus fumigatus*; *Aspergillus flavus*) on the surfaces of S3 heads and torsos and mostly torsos on S4. The analysis of small scavenger succession further exhibited preference to intestines and was highest with S3 and S4. Regression and correlation analyses demonstrated a significant positive correlation between temperature and weight loss for all carcasses and body parts ranging from r+0.90, $r^2= 0.81$ to r+0.91, $r^2= 0.82$, with intestines of S3 yielding a perfect correlation (r+1.00, $r^2=1.00$). Statistical analysis suggested that relative humidity would also be appropriate to be used as a variable to detect the relationship with rates of decay ranging from r=0.87 to r=0.94 for most body parts.

The results in this study demonstrate significant differences between the tested types of mutilation. The variations concerning the rate of decomposition were due to the internal and external microbial processes between different types of dismemberment caused by sharp force trauma, and aided by temperature and humidity variables, and entomological and small scavenger succession. These preliminary data will be useful for further research for assessing the PMI of the most frequent types of criminal mutilation in a terrestrial environment.

**Mutilated Body Parts, Postmortem Interval, Taphonomy**

## H7 Living With Corpses: Case Report of Psychological Impairment and Neglect, Leading to the Death of Two Women

*William C. Rodriguez III, PhD\*, Armed Forces Medical Examiner, 1413 Research Boulevard, Building 102, Rockville, MD 20850*

After attending this presentation, attendees will possess a better understanding on the importance of scene and behavioral evidence in the examination of mummified remains in which there is no clear anatomical evidence relating to cause and manner of death.

This presentation will impact the forensic community by educating attendees on the examination of mummified remains and various testing methods which may be utilized to possibly determine cause and manner of death. Furthermore, the presentation will focus on the psychological aspects of individuals who co-habitat with remains of the deceased, and the use of entomological and other evidence to determine the length of co-habitation with the dead.

In June of 2004 the mummified and partially skeletonized remains of a 56-year-old Japanese mother and her 33-year-old daughter were discovered in a mobile home in south eastern Idaho. The partially preserved remains were located lying next to each other in a bed with blankets and sheets positioned neatly up to the level of their chest. Appearance of the women which included their position next to each other, clothing, and intact styled hair with hair bands gave the impression that both died naturally in their sleep.

The decompositional stench of the bodies was so strong within the mobile home that investigators had to wear masks including oxygen packs. Upon continued examination of the scene, investigators noted that another individual had continued to live at the residence, that being the husband/father of the deceased. Several dozen solid, air deodorant devices were found throughout the mobile home. The deodorizing devices were noted on multiple tables and shelves in every room, in hallways, and within all of the air vents located in the floor of the mobile home. In addition to the solid air deodorizers, multiple cans of various aerosol based deodorizers were found scattered around the residence.

The husband/father of the deceased was later picked up by police as a possible suspect in the deaths. Interviews of the suspect revealed that he was a senior engineer at local nuclear plant, who also possessed a high level security clearance in relation to his employment. Interviews with his employer also revealed the subject to have received graduate degrees in engineering and science from Cornell University.

Forensic examination of the mother found her to be in a completely mummified state. No evidence of abrasions, bruising, skin discoloration or other injury was discovered upon external examination of the remains. Internal examination of the body revealed the presence of decomposed remnants of the lungs, kidney and liver. Examination of the inner skull revealed no evidence of tears or hemorrhage to the dried remains of the dura. A small amount of highly decomposed brain and no fractures were present on the inner as well as the outer table of the skull. Dissection and inspection of the mummified tissues of the neck revealed no evidence of hemorrhage or significant discoloration. Similar findings were noted upon examination of the trachea, and the hyoid and ossified cartilages of the neck were undamaged. No injuries were found on any of the postcranial elements. Toxicology findings on the recovered organs were negative.

Forensic examination of the daughter found her to be in an advanced state of skeletonization with partial mummification. Significant mummified epidermal tissue covered the anterior plane of the body with skeletonization primarily observed on the posterior plane. The only evidence of damage to the mummified epidermal tissues was in the area of the right shoulder and left abdominal area, which was identified as the result of carrion insect activity. The internal chest wall, including the ribs and costal cartilages, could be viewed from the back of the deceased due to the lack of soft tissues. No tears or separation of the ribs and costal cartilages were noted and there was no evidence of discoloration of the internal chest wall suggestive of hemorrhage. No discernable tears, abrasions, or discolorations were present on the mummified epidermal tissues of head. The skull was absent of fractures or other injuries and the same was true for the postcranial skeleton. Based on the mummified state of the deceased and the absence of blow fly activity, death was estimated to have occurred either in the winter of 2004 or 2003.

Continued investigation by police revealed the daughter would lock herself in her room when her father was in the house and that the father last reported seeing the daughter alive in April of 2001. Diary entries in April of 2001 by the wife make reference to the daughter needing to eat. A later entry by the wife notes the screaming sound of killdeer (a common bird species) and the awful smell of something dead in the house. Later the father admitted that in June of 2001, as the result of a bad smell, he broke into his daughter's room and found her dead and decomposing on the bed. Later in February of 2003, the husband found his wife dead, placed her next to his daughter, and pulled the blanket and covers up neatly. The suspect never informed anyone of the deaths, and continued to live at the house and to go to work daily.

Although homicide could not totally be ruled out in this case, the absence of external and skeletal trauma to the remains of the deceased and negative toxicological findings lead to the conclusion that death possibly resulted from either illness or starvation, or a combination of both. In either possible case, medical attention was not sought by the mother, daughter, or the husband/father. The psychological state of the mother and daughter may have well contributed to their demise; however, the burden of care fell to the responsibility of the husband/father. In conclusion, the husband later pleaded guilty to two counts of involuntary manslaughter.

**Mummification, Psychological, Scene Investigation**

## H8 Creating an Open-Air Forensic Anthropology Human Decomposition Research Facility

*Jerry Melbye, PhD\*, and Michelle D. Hamilton, PhD, Texas State University-San Marcos, Department of Anthropology, 601 University Drive, San Marcos, TX 78666-4616*

After attending this presentation, attendees will have a better understanding of the problems and limitations associated with establishing an open-air human decomposition facility.

This presentation will impact the forensic community by explaining the need to establish more open-air human decomposition stations in North America. This presentation will demonstrate to the scientific community that through dogged determination and lessons learned, it can be done.

Over twenty-five years ago, Dr. William M. Bass created the first formal open-air decomposition facility at the University of Tennessee. He realized that more information about time since death estimations was needed. The Tennessee facility continues to serve as a major contributor to knowledge of human decomposition rates to this day. Many researchers, including Dr. Bass, have noted that more of these facilities are needed to help more accurately understand the postmortem interval in different environmental conditions. Many researchers have tried to start a facility of this kind, but nearly all have failed, most as a result of lack of administrative and/or community support. Two recent success stories include Western Carolina University and Texas State University-San Marcos. The San Marcos facility serves as the focus for this paper. It has taken four years of hard work and doggedness to establish the facility at Texas State University, where a number of lessons have been learned along the way, all of which will be outlined in this paper as encouragement and a path for others to follow.

Depending on the university viewpoint, this may be the first hurdle to overcome: enthusiasm must be shown for the end result to gain full support of the Chair, the Dean, the Provost, and others high-up in the administrative echelon. Without administration support, the project will be abandoned before it can even begin. Once one understands what administrators want to hear, the "soft sell" can begin.

Gaining support from the law enforcement community can be another difficulty. By offering workshops, it will establish to law enforcement that the university is a valuable place for training and to university administrators that the project is a source of revenue. Now the argument can be made that a facility would generate even more income for research!

Public reaction can be a major issue that must be overcome at the onset. By and large, the public will support the project as long as it is located far away from their home. Therefore, the facility location may have to be a compromise between where you want it and where you can build it. It is imperative that the facility be located in an isolated area with an unoccupied buffer zone between the facility and the public.

Individuals who are not familiar with research involving human decomposition will have to be educated on how to differentiate between fact and fiction. Most people are concerned about wafting odors, disease-carrying flies, birds dropping body parts on doorsteps, and decreased property values. Simple, straightforward, honest answers at public forums can be offered to satisfy all of these questions. A very large concern from the public in the case of Texas State was the idea that decomposing bodies would pollute the environment. The response to this issue has led Texas State University to offer a new perspective on this environmental issue by turning this specific area of concern into a center-piece of the program; environmentally sound research and laboratory practices, and an eco-friendly solution to traditional funeral burials.

There is a need to establish more open-air human decomposition stations in North America. This presentation will demonstrate to the scientific community that through dogged determination and lessons learned, it can be done.

**Decomposition Facility, Time-Since-Death, Forensic Anthropology**

## H9   Metacarpal and Metatarsal Histology of Humans and Black Bears

*Brannon I. Hulsey, MA\*, Walter E. Klippel, PhD, and Lee Meadows Jantz, PhD, University of Tennessee, Department of Anthropology, 250 South Stadium Hall, Knoxville, TN 37966-0720*

After attending this presentation, attendees will understand the histological differences in the metacarpals and metatarsals of human hands and feet and black bear (*Ursus americanus*) paws.

This presentation will impact the forensic community by aiding in the separation and identification of morphologically similar osseous human and nonhuman remains.

The similarities between the bones of human hands and bear paws have been noted by numerous authors, and the morphological similarities and differences have been described extensively. While useful when whole bones are discovered, gross morphological characteristics may fail in the context of damaged or fragmented bones. In these situations, an alternative method of identification is necessary. Histology has been used to describe both human and nonhuman bones, and can be employed in separating bones as similar as those in this study. The histology of bear bones has been limited to femur and tibia midshaft cross sections, so descriptions of bear metapodials are needed in order to differentiate them from metacarpals and metatarsals in humans.

The human sample consists of two unprovenienced feet and an unprovenienced hand from the University of Tennessee's Anthropological Research Collections. The bear sample consists of sixteen paws from four bears (eight front and eight back) obtained in Minnesota, Wisconsin, Maine, and Tennessee, one back paw obtained in Georgia, one front paw obtained in Wisconsin, and a back paw that came in as a forensic case to the University of Tennessee, giving a total of 9 front paws from 5 bears and 10 back paws from 6 bears.

Cross sections were made at midshaft of all bear metapodials and in 5 mm increments proximally and distally from midshaft on the second metapodials of one front paw and one back paw from a single bear. This allows for examination of variation both within and across metapodials in a single bear and across metapodials in multiple bears. Cross sections were also made at midshaft of all human metapodials and in 5 mm increments proximally and distally from midshaft on the second metapodials of one hand and one foot. All thin sections were cut 15 µm thick and ground. Slides were viewed using a light microscope and photographed using the computer program ImagePro Express.

Several quantitative variables were examined in order to determine the difference between human and bear metapodials at the histological level. Quantitative measurements included maximum osteon diameter (µm), osteon area (µm²), maximum Haversian canal diameter (µm), and Haversian canal area (µm²). One to four osteons were measured per thin section and the values averaged for each species. The means were then tested using ANOVA to see if they differed between human and bear. In addition, the percentage of overlap in osteon and Haversian canal sizes was calculated between the two species. The incidence of several qualitative features was also noted when encountered, including osteon banding, resorption spaces, and plexiform bone.

Results show that human osteons and Haversian canals are larger in both diameter and area than those found in bears. The mean human osteon area and diameter are 39,081 µm² and 249 µm, respectively, while the mean bear osteon area and diameter are 21,421 µm² and 183 µm, respectively. The mean human Haversian canal area and diameter are 2,160 µm² and 58 µm, respectively, while the mean bear Haversian canal area and diameter are 580 µm² and 29 µm, respectively. In addition, the qualitative features of osteon banding, resorption spaces, and plexiform bone are more prevalent in bear metapodials than human metacarpals and metatarsals. These results indicate that it is possible to differentiate between fragmented bear and human metacarpals and metatarsals using a combination of qualitative and quantitative microscopic features.

**Black Bear, Human, Metacarpals/Metatarsals**

## H10   The Effects of Papain and EDTA on Bone in the Processing of Forensic Remains

*Bobbie J. Kemp, MS, Michael I. Siegel, PhD, Margaret A. Judd, PhD, and Mark P. Mooney, PhD, University of Pittsburgh, Department of Anthropology, 3302 Wesley W. Posvar Hall, Pittsburgh, PA 15260, and Luis L. Cabo-Pérez, MS, Mercyhurst College, Department of Applied Forensic Sciences, 501 East 38th Street, Erie, PA 16546*

The goal of this presentation is to assess the effects of papain and EDTA on bone as well as determine an effective, cost-efficient concentration of papain for the removal of soft tissue from bone.

This presentation will impact the forensic community by providing a method for soft tissue removal that is quick, efficient, and nondestructive to bone.

It is essential in forensic anthropology to employ a defleshing method that is both efficient and nondestructive to bone tissue or fine trauma marks, while working within time constraints imposed by law enforcement. Results from a previous study (Kemp et al. 2008) suggested that papain solutions fulfill these conditions, representing a viable method which can be utilized in forensic contexts. However, other studies have found that papain can be destructive to bone.

Papain is a proteolytic enzyme (protease) derived from the unripened papaya fruit (*Carica papaya*). Proteases are specific enzymes that induce protein decomposition (proteolysis) by promoting hydrolysis of the peptide bonds that link amino acids. Therefore, these enzymes would primarily target the protein content of muscle and connective

tissue (ligaments, tendons, and cartilage), rather than the mineral or tightly packaged organic matrix of the bone. Other benefits of the enzyme are that optimal activity occurs at low temperatures (45°-65°C), thus remains need not be subjected to boiling, and it works so effectively that no harsh instrument is required to remove soft tissues.

Papain can be inactivated by metal ions, so commercial papain solutions normally contain ethylenediamine tetra-acetic acid (EDTA), as a chelating agent. EDTA binds to metal ions, including calcium, forming metal complexes with very high stability constants (pK-values). Thus, EDTA can bind to and remove calcium from bone tissue. Due to this property, EDTA is commonly used to decalcify bone for the preparation of histological slides or the retrieval of DNA. Therefore, it is proposed that the destruction of bone tissues reported in other studies may in fact be attributed to EDTA rather than papain.

The present study addresses three primary questions: (1) What is the most effective, cost-efficient concentration of papain for soft tissue removal? (2) Is papain destructive to bone at this concentration? and (3) Is EDTA destructive to bone?

A sample (n=16) of New Zealand white rabbits (*Oryctolagus cuniculus*) was obtained from a colony housed at the University of Pittsburgh (all individuals in the study came from previously approved protocols). The skulls, forelimbs, and hindlimbs of each rabbit were removed following standardized procedures. All remains were weighed prior to and following processing, recording any differences in wet or dry weights. The subsequent steps of the study were conducted in two phases. The first phase of the study focused on the effectiveness of papain and its effect on bone, while the second focused on the effect of EDTA on bone.

Phase 1 samples (12 forelimbs, 15 hindlimbs, and 11 skulls) were placed in stainless steel pots in solutions consisting of 10g, 8g, 6g, 4g, 2g or 0g (control group) of papain in $3.8 \times 10^3$ ml (1gal) of distilled water. Solutions were heated on electric burners, then heated to and maintained at 55°-65° C. The condition of the remains was documented every 30 minutes. Results suggested that the 4g solution was the most efficient and cost-effective, based on variables such as the time-to-completion, final dry weight of the bones, and efficacy of the enzyme on varying tissue types. Hence, this concentration was employed in the EDTA solutions analyzed in Phase 2 of the study.

Phase 2 samples (7 forelimbs and 6 hindlimbs) were placed in solutions consisting of $3.8 \times 10^3$ ml (1 gal) of distilled water, 4g papain, and 10g, 6g, or 2g of EDTA. The same procedures were followed as described above in Phase 1.

Gross bone destruction was not noted in any of the Phase 1 samples, whereas severe destruction was observed on several bones in the Phase 2 EDTA samples. The thin bone of most of the scapulae was eroded through, and some subchondral bone was also destroyed. These results strongly suggest that EDTA, rather than papain, is highly destructive to bone and should be avoided in defleshing protocols. The developed protocol reduces the amount of free metal ions in the solution by using distilled water rather than tap water and stainless steel pots rather than aluminum, therefore, EDTA is not required for proper activity of papain. The enzyme was found to work quickly and effectively with the proposed protocol, without EDTA significantly affecting the time-to-completion.

**Reference:**

[1] Kemp BJ, Cabo-Perez LM, Matia J, Dirkmaat DC. 2008. The effectiveness of papain in the processing of remains. Poster presented at the 60th annual meeting of the American Academy of Forensic Sciences.

**Maceration, Papain, EDTA**

## H11 Practical Considerations in Trace Element Analysis of Bone by Portable X-Ray Fluorescence

*Jennifer F. Byrnes, BS\*, SUNY University at Buffalo, Department of Anthropology, 380 MFAC, Ellicott Complex, Buffalo, NY 14261-0026; and Peter J. Bush, BS, SUNY at Buffalo, South Campus Instrument Center, B1 Squire Hall, South Campus, Buffalo, NY 14214*

The goal of this presentation is to discuss analysis parameters in the use of portable x-ray fluorescence (XRF) in the analysis of trace element levels in human bone.

This presentation will impact the forensic community by providing information on possible error factors in XRF analysis of bone.

Bone chemistry can reveal much about an individual's life history. Certain trace elements can give specific and pertinent information about environmental exposure, diet, and geographical location of residence. X-Ray Fluorescence is a nondestructive method for analysis of trace elements. Portable XRF instruments now available allow trace element analysis of samples in the field or in collections. The use of such instruments requires knowledge of analysis parameters such as x-ray penetration and exit depth. This is especially important if non-homogenized whole bone samples are to be analyzed. In a forensic or burial setting where the bone sample has been exposed to the environment, the elements being studied may be altered due to diagenetic changes. Depending on a variety of factors such as soil and ground water composition, the observed reading may not fully represent the actual bone chemistry composition of the individual. The structure of cortical bone is another important factor because the newly deposited bone layers on the outer surface may have different values of a given element than an older deposited layer deep to the surface, as bone undergoes constant remodeling throughout one's life. Cortical bone is more conducive to use with this portable XRF, as trabecular bone has a number of inconsistencies such as an unsmooth surface, which can inhibit the analytical performance of the procedure.

Two experiments were carried out to understand how analysis depth and removal of surface layers of bone affect analytical results. Analysis depth was determined by examining selected pure elements through varying known thicknesses of cow tibia cortical bone slices. This showed a correlation between the element's x-ray emission energy and the depth of reading by the device. In the second experiment, a small human population from an unknown graveyard found in Youngstown, NY was studied. The burials were discovered and excavated in 1997; very little is known about who these individuals were other than what the osteological analysis has revealed, as few artifacts were recovered from the graves. The surfaces of the bones from this collection were analyzed before and after sanding, to observe if sanding the immediate surface of the analyzed area alters the results. In a burial setting, the way the bones were positioned in the grave can affect the readings taken from one side of the bone to the other. This may result from contact with soil or ground water intrusion in the gravesite. This research has concluded that it is important to take these factors into consideration when performing bone analysis. When determining what areas of the bone to analyze for a particular element under study (a skull fragment versus a long bone fragment) cortical bone thickness and the emission energies of the element of interest should also come into consideration. These results validate the portable XRF device as a powerful and convenient instrument for nondestructive analysis, while highlighting a number of limitations and considerations for its use in obtaining data from bone samples.

**Portable XRF, Trace Elements, Bone**

## H12 Field Contamination of Archaeological Bone Samples Submitted for Mitochondrial DNA (mtDNA) Analysis

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After attending the presentation, attendees will understand the importance of field and laboratory procedures that minimize the potential for DNA contamination, and will be briefed on analysis procedures to enable detection of contamination that does occur.

This presentation will impact the forensic community by illustrating how DNA samples may be contaminated in the field even when stringent laboratory procedures are followed to avoid such contamination. It will also demonstrate how such contamination may be detected.

The Armed Forces DNA Identification Laboratory (AFDIL) has been generating mitochondrial DNA (mtDNA) profiles from the osseous remains of missing U.S. service members and civilians in support of the mission of the Joint POW/MIA Accounting Command–Central Identification Laboratory (JPAC-CIL) since 1992. Extensive precautions are taken in order to assure that the sequences generated are authentic and not that of an outside or modern contaminant.

Once remains are accessioned at the JPAC-CIL, they are housed in secure storage and checked out to a specific analyst for each stage of analysis. All analysts wear personal protective equipment (PPE) during analysis to reduce the risk of contamination. Remains are sampled for mtDNA analysis as soon as possible after accessioning. Samples are obtained using single use rotary blades that have been sterilized in 20% bleach solution and with an ultraviolet crosslinker, and bleach and ultraviolet light are applied to the interior of the sampling hood prior to each sample being cut. Samples are individually bagged and sent to AFDIL for analysis.

At AFDIL, samples are similarly accessioned and checked out for analysis. Details of internal protocols at AFDIL can be found in Edson, et al., 2004. Methods taken to avoid contamination include, but are not limited to, the following: the removal of the outside surface of the bone by sanding and subsequent washing in $diH_2O$ and EtOH, preparation and extraction of the samples in "clean" rooms using individual hoods, and the wearing of multiple layers of PPE. Profiles are generated from the extracts using a redundant amplification strategy of overlapping primers and a subsequent comparison to an internal database of staff profiles, including both AFDIL and JPAC-CIL staff, in addition to any laboratory visitors. Profiles generated from remains are not reported until they meet the internal criteria of multiple, consistent amplifications and are proven not to be consistent with any staff members at either laboratory who had a potential exposure to the samples (as tracked by the laboratories' computerized accession records).

While extensive care is taken once the remains are received at JPAC and AFDIL, there is limited control of possible contamination events in the field while remains are being recovered. On average, over 750 osseous samples are processed at AFDIL each year, and such field contamination events have proven to be rare. However, in recent years, two field contamination events have occurred. Both of these cases involved historical aircraft crash sites, in which the anthropologist directing the recovery contaminated a bone fragment in the field. In the first case, five samples were submitted to AFDIL for processing. Three of the samples were reported and were consistent with the two individuals presumed to be on the aircraft. No data was generated from one sample; but the fifth sample showed a mixture of the endogenous sequence (consistent with one crewmember) and that of the field anthropologist. The sample was exceptionally small, only 1.1 g, and cut into two pieces upon submission. In the second case, many of the bone samples were burned. One of the samples produced a high-quality sequence that appeared to be endogenous and met the reporting criteria. However, upon comparison to the sequence database, it was found that this profile was consistent with the field anthropologist and inconsistent with references for any of the crewmembers, and the sample was not reported.

These two cases demonstrate the importance of selecting samples of good quality and of sufficient size for cleaning. While the cleaning protocols are certainly more than adequate under normal conditions, both of these samples had limitations which prevented the cleaning protocol from being sufficiently applied; the first sample being very small and difficult to hold during sanding and the second having been subjected to burning and therefore having an extremely friable surface. Smart sample selection, coupled with reduced handling of samples in the field will help to eliminate possible contamination events and/or false reporting of results.

The above described events occurred in 2006 and 2007 respectively. While other contamination events have occurred at a low-level, these are the only two instances of the field anthropologist introducing modern DNA to the entire sample, a failure rate of 0.14% and 0.12%, respectively. This presentation will focus on the details of the protocols used at AFDIL and JPAC-CIL for the processing of degraded osseous remains. Attendees will learn how to perhaps implement these protocols into their own laboratory use for prevention and detection of field and in-house contamination of mtDNA samples.

The views expressed herein are those of the authors and not necessarily those of the Joint POW/MIA Accounting Command, the Armed Forces Institute of Pathology, the US Army Surgeon General, nor the US Department of Defense.

**Reference:**

Edson, S.M., J.P. Ross, M.D. Coble, T.J. Parsons, and S.M. Barritt (2004). Naming the Dead: Confronting the Realities of the Rapid Identification of Degraded Skeletal Remains. Forensic Science Review **16(1)**: 63-90.

**Mitochondrial DNA, Contamination, Human Skeletal Remains**

## H13 Training in Forensic Archaeology and Anthropology on a Shoestring: Is It Possible? Is It Sensible?

*Roland Wessling, BSc\*, and Ambika Flavel,* MSc, *Inforce Foundation, Cranfield University, Cranfield Forensic Institute, Shrivenham, SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will have an increased understanding of the challenges and possibilities involved in providing training in forensic archaeology and anthropology around the world, as well as the various cost implications of such training.

This presentation will impact the forensic community by presenting a series of real examples that show the overall structure of operational budgets, as well as the individual aspects of planning training programs. The presentation will close with a series of conclusions and propositions which are aimed to trigger more discussion regarding funding and the cost of training and exercising in forensic archaeology and anthropology. This will, in turn, hopefully lead to more training programs.

Many countries have been, and still are, affected by atrocity crimes. Often such crimes have not been investigated enough or at all due to political, security, or other reasons. In some countries, for example Guatemala and Argentina, teams of forensic anthropologists have a tradition of investigating these atrocity crimes. These teams consist

mainly of scientists from that particular nation, although most teams have had some international individuals working with them for certain periods of time.  In contrast, countries such as Bosnia and Herzegovina, Croatia, and Kosovo have had international forensic teams investigating their atrocity crimes with national teams continuing the work later. Whichever model an investigation takes, the thorough training of all team members is of vital importance.

Every aspect of atrocity crimes investigations has a significant impact on many peoples' lives:  relatives of the missing who may learn what truly happened to their loved ones; nations that have to come to terms with what atrocities have been committed in their name; criminal court proceedings; insurance claims; etc.  The people who have been affected by atrocity crimes through the loss of relatives have experienced extensive emotional trauma.  Facing this reality, forensic teams must ensure that every process within their work is carried out to the highest, internationally recognized standards.  If a victim is misidentified and repatriated to the wrong family, the trauma the relatives have to go through is simply unacceptable.   It is therefore essential that all personnel working on atrocity crime investigations are properly trained in their specific tasks, have up-to-date and comprehensive knowledge of everyone else's roles, and completely understand the overall process and their place within it.  This involves significant theoretical knowledge as well as relevant and well-structured training and exercising.

There are a number of organizations in the world that have the expertise and experience to provide high quality training in atrocity crime investigations; and there are many countries that have a substantial need for such training.  Why then does so little take place?  The answer lies in part in the cost of such training.  Other factors, such as security problems or political interference, can rarely be addressed and never solved by forensic archaeologists or anthropologists.  The issue of costs, however, may be mitigated.

During this presentation, a series of real examples will be presented that show the overall structure of operational budgets, as well as other individual aspects associated with the planning of forensic training programs.   In conclusion, the presentation hopes to trigger more discussion regarding funding and the cost of training in forensic archaeology and anthropology such as:   Why is forensic training expensive?  Does it have to be?  Where can costs be reduced?  Where can they not be?  This will, in turn, hopefully lead to the creation of more training programs.

**Forensic Archaeology, Forensic Anthropology, Training and Exercising**

# H14    Ground Penetrating Radar:  A New Tool in Crime Scene Examination?

*Donna M. MacGregor, MSc\*, Queensland Police Service, Scientific Section, 200 Roma Street, Brisbane, 4001, AUSTRALIA*

After attending this presentation, attendees will understand the search techniques employed to locate buried bodies or items within Queensland, Australia, and how new techiques are being evaluated.

This presentation will impact the forensic science community by illustrating the role that the Queensland Scientific Police serve and the equipment and techniques utilized to locate various buried items.  These techniques are now being evaluated in Queensland in order to offer better service delivery to plain clothes investigators.

Recently the Queensland Police Service (QPS) considered the purchase of a ground penetrating radar (GPR) system.  The current practice within the QPS with regards to GPR is to employ the services of a private operator and GPR system on a case-by-case basis.  As the call for GPR services increases, it may be more cost effective for the QPS to acquire the GPR equipment and skill base as a service resource.

GPR operates by radiating short pulses of energy into a medium, usually soil, via a transmitter antenna and then receiving the energy reflected back from geological, or forensic, features with varying electromagnetic properties.  By recording the propagation time delay between the transmitted and received signal, the distance to subsurface features such as rocks, tree roots, and objects of forensic interest can be determined.

GPR has many benefits compared with traditional search techniques.  GPR is nondestructive, relatively portable, self-contained, and can readily provide high resolution data in real time on the radar display unit.  The availability of real time data provides investigators with vital information about subsurface features while in the scene and greatly assists the forensic personnel by identifying specific areas of interest for excavation.

The use of GPR by law enforcement agencies is extensively documented in the forensic literature; however, most of this literature is derived from international experiences.  It is not a tool routinely utilized within Australia for crime scene examinations.  Prior to the purchase of any GPR unit, validation trials were required to be conducted.

Several burials sites were established based on simulated crime scene scenarios.  These burial sites were positioned beside each other in a row.  The scenarios represented included:
1.  Non-disturbed soil (Control),
2.  Disturbed soil to 300mm with no object,
3.  Disturbed soil to 500mm with no object,
4.  Disturbed soil to 300mm with mixture of plastic skeleton and bovine skeletal elements, and
5.  Disturbed soil to 300mm with metal weapon.

The GPR unit utilized in the trials was the GSSI SIR-3000, loaned from the Commonwealth Scientific and Industrial Research Organization (CSIRO) Mining Automation.  Two antennas were trailed in association with this system.  These were the GSSI 900MHz and GSSI 400MHz antennas, both of which are interchangeable with the SIR-3000 system.

The various burial sites were contained within an area approximately 4m x 8m.  During the first trial an X-Y grid was established for a survey pattern.  A series of runs or scans were conducted at regular intervals along the X-axis and then along the Y-axis.  A total of 27 scans were obtained for the study area.  Each scan provided information on subsurface items on the display unit.  Sites 2-5 were readily identified by the disturbances in the soil, represented on the display screen as hyperbola.  The raw data displayed a significant difference between the reflection of the metal weapon (Site 5) and all the other sites, with the metal weapon displaying a stronger hyperbola.  The raw data also displayed a difference between Site 2 and Site 3, with Site 3 exhibiting a disturbance further into the soil than Site 2.  Interestingly the scans obtained during this trial could be refined at a later time during subsequent processing to provide 3-D images of the study area.  These 3-D scans will be presented during the presentation.

A second trial was conducted using the same burials pits however 30mm cement pavers were placed on the soil surface above each burial site. The GPR was subsequently run over each burial site again using the same X-Y grid pattern.  The results displayed during this trial were similar to the results of trial 1 in terms of the differences between Sites 1-5 discussed above.  The concrete pavers over the soil surface simply added an additional feature within each scan.   These pavers were reinforced with steel which is represented in each scan as regular spaced dots along the top of the images.  The disturbed soil below the concrete pavers was still able to be observed as hyperbolas.  The metal weapon in Site 5 again produced the strongest hyperbola.

The 400 MHz antenna provided the best resolution for the visualization of the subsurface features compared to the 900 MHz antenna.  The 400 MHz antenna is commonly referred to in the literature as the antenna of choice for burial work.   The results of the trial concurred with this observation.

**Crime Scene, Recovery, Search Techniques**

## H15	Forensic Field Radiography: In the Trenches With MacGyver

*Gerald J. Conlogue, MHS\*, c/o Diagnostic Imaging Program, Quinnipiac University, 275 Mt. Carmel Avenue, Hamden, CT 06518; and Mark D. Viner, MSc, Inforce Foundation, Forensic Science Institute, Cranfield University, Royal Military College of Science, Shrivenham, Wiltshire, UNITED KINGDOM*

After attending this presentation, attendees will learn how radiological imaging is a very useful tool in forensic and archaeological investigation. However it is often not deployed in circumstances where it would be very useful due to perceived practical, financial, and logistical concerns. This presentation will demonstrate that with prior planning, many of these concerns can be easily overcome and that a field radiography facility can be swiftly and economically established even in remote areas.

This presentation will impact the forensic community by demonstrating how radiography is a very useful tool in forensic investigation. With prior planning it can easily be deployed in field conditions, offering the opportunity to gather and preserve evidence in difficult or complex situations.

**Hypothesis:** The potential value of obtaining radiological images using state-of-the-art facilities in hospitals and established research centers cannot be denied. However, access to such facilities is not always possible or desirable. In such circumstances, employing conventional radiography in a field situation offers a viable alternative, particularly in anthropological and forensic applications.

**Methods & Results:** Establishing a radiographic facility at or close to the site at which remains are recovered and/or stored has a number of advantages. These include: (1) minimal disruption of the taphonomic context, (2) minimal disruption of foreign bodies and/or artifacts that could potentially compromise the evidential value of the data, (3) radiography can be used to triage remains in order to select those for transportation to an imaging facility with advanced modalities such as computed tomography, (4) imaging can be performed in circumstances where transportation is impossible for physical or logistical reasons, and (5) in the case of sensitive human rights situations, there may be security considerations preventing transportation, and/or making it difficult to secure a separate mortuary facility.

Conventional radiographic equipment is highly mobile and a field radiographic facility can be easily established even in remote areas provided that a number of factors are taken into consideration. The objectives of the study or investigation must first be clearly defined, detailing the number and type of specimens to be examined and the aim of such examinations. An experienced radiographer should then develop the radiographic design of the project in conjunction with the investigating team. Project design will include: (1) development of the imaging protocol and selection of appropriate radiographic unit and image recording method, (2) site survey and risk assessments, (3) facility design including x-ray, image processing, image storage and viewing facilities, (4) facility construction, (5) development of schemes of work and health and safety rules, (6) development of a quality control plan and procedures, and (7) facility commissioning including staff familiarization and training.

**Conclusion:** Radiological imaging is a very useful tool in forensic and archaeological investigation. In certain situations the establishment of a field radiography facility may offer significant benefits to the investigation and should not be discounted on logistical, organizational or financial grounds without a feasibility study being concluded by an experienced radiographer.

**Field Radiography, Forensic Radiography, Mass Fatality Incidents**

## H16	Hispanic: History and Use of a Generic Term

*Ashley E. Shidner, BA\*, and Heidi S. Davis, BA, BS, University of West Florida, Department of Anthropology, 11000 University Parkway, Pensacola, FL 32514*

The learning objective of this presentation is to discuss the basic definitions of social race and ethnicity as they relate to the term Hispanic.

This presentation will impact the forensic community by discussing the history of the term Hispanic and its common use as a social race and ethnic category.

Using the basic definitions of social race and ethnicity as they relate to the term Hispanic, this presentation will demonstrate that the term Hispanic does not fit into either category, further perpetuating confusion regarding use of the term.

Hispanic is a term that was adopted and poorly defined by the United States government in order to address a minority population comprised of various national origin groups. The term Hispanic is thought to be a derivation of the Latin word *Hispania,* used to refer to region of modern day Spain or the Iberian Peninsula by the Romans (Marín and Marín 1991). When Spanish settlers arrived in New Mexico they were referred to as Hispanos. This term was later rejected in the southwest after Mexico won independence from Spain in lieu of the term Latino, because it rejected the Spain of the conquistadors (Melville 1988). In 1968 congress authorized President Johnson to declare the week including September 15 and 16 as National Hispanic Heritage Week. It was after this declaration the term Hispanic became more widespread.

Based on a 1977-1978 government order, Hispanic was defined as "a person of Mexican, Puerto Rican, Cuban, Central, South American, or other Spanish culture or origin, regardless of race" (Forbes 1999:62). This definition distinguished Hispanic as an ethnicity, attempting to suggest a common cultural or language origin, rather than a racial category. According to Melville (1988), the most common practice for an individual is to refer to themselves as based on their national origin. However, in official government reporting, the U.S. Census Bureau considers Hispanic as an ethnicity to be selected in conjunction with a racial category of Black or White. Thus, while many individuals from Spanish-speaking countries would *not* refer to themselves as Hispanic in their native country, they are forced to do so in the United States.

Despite governmental mandates and personal choice, Hispanics are continually referred to as both a racial and an ethnic group. Further, these terms are often used interchangeably despite significant differences between their meanings. Social race in the United States is based on skin pigmentation and other phenotypic characteristics that are believed to reflect ones ancestral genealogy. However, these phenotypical characteristics continually fail to "identify" Hispanic individuals. For example, in Puerto Rico, many individuals would be racially classified as White or Black in the United States, or even a third racial category within their own country (Rodríguez 2000).

Race and ethnicity can be differentiated on the idea that ethnicity is strictly based on the idea that the group of individuals share some cultural ancestry or heritage. Brazilians, who speak Portuguese, and Guatemala Mayans, who speak unique Mayan dialects, are often referred to as Hispanic in the United States. The discordance of language and the unique population histories of these groups reject the U.S. government's designation of Hispanic of belonging to a shared Spanish culture or origin. Due to confusing and misguided terminology, it is not surprising that anthropologists are not able to define this term accurately due to the conflicting differences between self-identification and governmental identification. This presentation concludes that when conducting forensic anthropological research using Hispanic individuals, anthropologists must clearly define their population groups, using

national origins, language, and self-described identity. Defining population groups will enable a better understanding of the unique population groups that are often referred to as Hispanic.

**Hispanic, Race, Ethnicity**

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## H17 Cephalic Index of Gurung Community of Nepal: An Anthropometric Study

*Stany W. Lobo, MSc\*, Department of Anatomy, Melaka Manipal Medical College, Manipal, Karnataka 576104, INDIA*

After attending this presentation, attendees will understand why the Gurung community of Nepal is classified as brachycephalic.

This presentation will impact the forensic community by introducing the first study on Cephalic Index of the Nepalese Gurung community.

Morphological differences between people can be recorded by measurements and based on these measurements different indices are calculated. Cephalic Index (CI) is one such index that can be used in differentiation of racial characteristics. Comparison of CI between parents, offspring, and their siblings can give a clue towards the genetic transmission of inherited characteristics. Cephalometry is also important in forensic science for facial reconstruction of disputed identity.

Nepal is a land-locked country with China and Tibet on its north and India on its south. The people of the Gurung community are the original inhabitants of the Himalayan Republic of Nepal and maintain tradition, customs, and social order of ancient Nepalese. This is the first cephalometric study of Gurung community of Nepal. A comparison of the CI of the Nepalese Gurung community with the studies done elsewhere is made. The present cross-sectional study was conducted in Gandhruk village of Western Development Region of Nepal which is predominantly inhabited by Gurung community. Individuals with any craniofacial abnormality were excluded from the study.

All the measurements were taken with the subjects sitting on a chair with their head in the anatomical position. The measurements were taken to the nearest mm using a spreading caliper. The anatomical landmarks considered for measurement of the head length and head breadth were glabella, inion, and euryon. The head length was measured from glabella to inion. The head breadth was measured as the maximum transverse diameter between the two euryons. Data thus obtained were computed and analyzed using SPSS (Statistical Package for Social Sciences) version 10. The CI was calculated using the formula: (head breadth/head length) x 100. The differences in means of head length, head breadth, and CI between male and female were tested for statistical significance by independent sample "t" test. A total of 267 adults comprising of 157 (58.8%) males and 110 (41.2%) females in the age range 25 to 45 years were studied. The mean head length of the whole sample was 17.7 cm (SD, 0.88). The mean head breadth of the sample was 14.9 cm (SD, 0.76). The mean CI of the sample was 83.7 (SD, 5.69). For males, the mean head length was 18 cm (SD, 0.85) and the mean breadth was 14.9 cm (SD, 0.83). For females, the mean length and mean breadth were 17.4 cm (SD, 0.78) and 14.7 cm (SD, 0.6), respectively. The mean CI for males and females was 83.1 (SD, 6.08) and 84.6 (SD, 5.14) respectively.

Attendees will learn from the results that the Nepalese Gurung community can be classified as brachycephalic. In tropical zones the head form is longer (dolichocephalic), but in temperate zones the head form is round (mesocephalic or brachycephalic). Since Nepal is in the temperate zone, the present classification of the Nepalese Gurung community as brachycephalic is in accordance with the geographical variations of CI.

**Cephalic Index, Gurung Community, Brachycephalic**

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## H18 Ancestry Estimation Using the Femur: A Pilot Study

*Sarah E. McManus, BA\*, 2019 Stonybrook Road, Louisville, TN 37777*

After attending this presentation, attendees will understand the utility of metric analysis of the femur in the estimation of ancestry.

This presentation will impact the forensic community by assisting with the ancestry estimation of unidentified human remains.

Estimation of ancestry is an important part of the creation of a biological profile for unknown skeletal remains. As techniques for ancestry estimation have primarily focused on the cranium, reliable methods using postcranial elements are both few in number and underutilized. Craig (1995) presented a method of ancestry estimation utilizing measurement of the intercondylar shelf angle from radiographs. While this study presents highly accurate classification rates, Berg et al. (2007) found statistically significant differences for intra- and inter-observer error using this method, placing its reliability in question.

Craig (1995) states that intercondylar shelf angle correlates with intercondylar notch height, with an acute angle corresponding to a higher notch height and an obtuse angle corresponding to a lower notch height. Baker et al. (1990) investigated the determining power of intercondylar notch height, producing accuracy rates between 76.92% and 82.5% using sectioning points. In addition to adapting measurement of intercondylar notch height from Baker et al. (1990), Gill (2001) also describes differences in femoral platymeria and torsion (evaluated from measurements of subtrochanteric diameter and maximum head height) that are potentially useful in the estimation of ancestry.

In order to examine the validity of the femur as an estimator of ancestry using a suite of measurements designed to incorporate all potential variation, a sample from the William M. Bass Donated Skeletal Collection at the The University of Tennessee consisting of 36 individuals (10 white males, 10 black males, 10 white females, and 6 black females) was selected. Ten measurements designed to assess the intercondylar notch, variation within the distal femur, platymeria, and torsion were taken from the left femur of each individual.

Analysis was then conducted to determine the measurements producing the most statistically significant differences between the groups used in this pilot study. Based on regression analysis in NCSS (NCSS LLC) five measurements were selected in order to conduct linear discriminant analysis on the data. For females the measurements selected were intercondylar notch height, medial condyle width, lateral condyle width, transverse subtrochanteric diameter, and epicondylar breadth, while for males the measurements selected were intercondylar notch height, medial condyle width, maximum head height, antero-posterior subtrochanteric diameter, and epicondylar breadth. Preliminary results of linear discriminant analysis in SAS 9.1 (SAS INSTITUTE) using these measurements indicate cross-validated accuracy rates of 90% for males and 100% for females.

Based on the preliminary findings of this pilot study the femur appears to be of valid use in the estimation of ancestry and warrants further study.

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**Femur, Ancestry, Forensic Anthropology**

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## H19 Evaluation of Enamel Short Chemical History as a Forensic Tool: A Comparative Study of Six Countries

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The goal of this presentation is to present the method of enamel surface microsampling for acid etching for trace element analysis in the identification of an individual's geographical affiliation.

This presentation will impact the forensic community by introducing a non-destructive and quick sampling tool. It will also contribute to the body of literature concerning enamel surface trace element studies.

The enamel surface of teeth is complex: internally, it is highly chemically active (e.g., remineralization), whereas externally it reacts with the oral, or the burial, medium. Its inorganic chemical elements vary due to factors such as age, sex, oral cavity activities (e.g., food and drink) and environment. In forensic identification, it is important to examine the enamel surface both chemically and morphologically (e.g., microwear) for indicators of an individual's health and place of residence as well as taphonomic conditions which may be identified and reconstructed. There is, however, little research concerning the study of the enamel chemical history of individuals over a short duration.

Extracted tooth samples were collected from the United Kingdom (n=14), the United Arab Emirates (n=11), the Sultanate of Oman (n=11), Iraq (n=10), Yemen (n=15), and Iran (n=15). The sources of teeth were from dental clinics and from Iraq they were derived from the Medicolegal Institute in Baghdad. The Iraq sample included postmortem teeth from forensic cases which had been subjected to explosive and burial conditions. Teeth were washed with tap water and subsequently in distilled water. The enamel surface was sampled, using the acid etching method, by applying a perforated adhesive tape (with 2mm diameter circular hole) and etching that area of the surface with 5 µl of 1.6 N HCL in 70 % glycerol for 35 seconds. The biopsy solution was analyzed for trace elements by the ICP-MS system. Neither the tooth type nor site of sampling were controlled, rather this was dependent upon individual tooth features (e.g., caries, enamel surface cleanliness, smoothness). The elements analyzed included Li, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, Ba, and Pb and overlapped ratios between them. The relative prevalence of these elements was used to assess the variability between the groups. Enamel etching depth was calculated mathematically using the phosphorus concentration. The enamel was examined by stereo microscope and SEM-EDXA system.

Enamel etched depths resulting in high element concentrations were approximately between 5 µm and 35µm. Regression analysis indicated that 11 element concentrations were significantly ($p < 0.05$) related to the etched depth. The relationship was strong with Ca ($R2= 0.9$) but was weak with other elements ($R2= 0.5$ to $-0.04$), so this was ignored. Some elements distinguished between countries, such as As which has the highest range in the United Kingdom and the lowest in Iran; whereas the Sr range is highest in Yemen and lowest in the United Kingdom clearly reflecting the pollution (e.g., food, air and water) and dietary condition, respectively.

The concentration of the elements Al, Ni, and Se were found to be related significantly ($p<0.05$) to the sex of the individual and the concentration of the elements As, Ti, and Zn were found to be related significantly ($p<0.05$) to the age of the individual, whereas the elements Cr, Mn, Mo, and Pb were found to be related to both sex and age. Additionally, principal components analysis was used to analyze selected elements from the sample (Pb, Sr, Mg, Zn, Cr, As, V, and Ti) after treating them statistically using a compositional statistic method, which takes the log of each element when divided by the Ca concentration.

This method was able to separate the groups.

One of the particles adhering to Iraqi sample 10 was analyzed using EDXA and found to contain mostly metal, including Cr (4.69 %). This may have interfered with the result, as the Iraqi group has the highest range of Cr in a graphical comparison to the other groups. Confounding factors of this method include the sand, caries, calculus, and restorations. The method is useful to record the enamel surface chemical composition and may shed light on the geographical origin of an individual skeleton. Further study is needed to evaluate this method prior to routine usage with forensic casework.

**Enamel, Chemistry, Forensic**

## H20 Differentiating Between Foreign National Hispanics and U.S. Hispanics in the Southwest: The Influence of Socioeconomic Status on Dental Health and Stature

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After attending this presentation, attendees will learn how the Pima County (Arizona) Office of the Medical Examiner uses dental health and stature of certain decedents to aid in inferring socioeconomic status and likely foreign citizenship.

This presentation will impact the forensic community by illustrating how unidentified individuals can be classified as probable foreign nationals through an assessment of their dental health and stature.

Differentiating between U.S. Hispanics and foreign national Hispanics is of paramount importance to the Pima County (Arizona) Office of the Medical Examiner (PCOME). This is due to the large number of foreign national migrants who have died in the Sonoran Desert during the past seven years. Because the PCOME has identified slightly more than 800 of the 1,138 migrants whose remains were recovered between 2001 and 2007, biological profiles for many of these identified individuals have proved to be instrumental in helping characterize the over 300 unidentified individuals as likely migrants. Two aspects of the biological profile that have proved to be useful in distinguishing U.S. Hispanics in Arizona from foreign national Hispanics are stature and dental health. Crown-heel measurements taken during the postmortem examination comprise most of the data (n=742) for migrant stature, with mean stature estimates derived from skeletal elements rounding out the sample of nearly 800 individuals. Dental examinations performed during the more than 200 forensic anthropology examinations on known foreign national migrants comprise the second postmortem sample. Dental health characteristics evaluated include caries, abscesses, antemortem tooth loss, restorations, crowded teeth, alveolar bone loss, dental calculus, enamel hypolasias, and tooth wear. Dental health and stature were recorded from the known migrants and then compared to several databases of U.S. Hispanics. Several thousand Southern Arizona Hispanics who were examined at the PCOME between 2001 and 2008 comprise the first U.S. sample. The second U.S. sample comprises ethnically Mexican subjects (n=465 males; n=537 females) from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) for stature and dental health data from the Hispanic Health and Nutrition Examination Survey (HHANES). The final database is the U.S. Army's 2007 ANSUR II Pilot Study which includes stature data on 123 ethnically Mexican male soldiers and 28 female ethnically Mexican

soldiers. Results from the stature comparisons reveal that U.S. born Hispanics, at least those of Mexican heritage, are taller on average than migrants born in Mexico. U.S. foreign-born stature differences were the largest for civilian males (89.2 mm), with a mean difference of 24.7 mm for civilian females and 28.3 mm for Army males. The Army female sample included only three foreign born soldiers, and so its mean difference is not reported although it follows the same directional trend as the other samples. Results from the dental health comparisons reveal that U.S. Hispanics of Mexican heritage have better dental health than the Mexican Nationals who have died while attempting to migrate. It is postulated that because many of the deceased migrants examined at the PCOME come from impoverished areas of Mexico and Central America, their lower socioeconomic standing prevents them from have an optimum diet and from seeking adequate medical and dental care. Because U.S. Hispanics apparently have a better standard of living than this, their stature is greater and their dental health is better. One implication of these findings is that a "socioeconomic profile" can be generated from the unidentified individuals to gauge whether they are more likely U.S. citizens or foreign national migrants. This profile is one line of several that is utilized in classifying some unidentified individuals as probable migrants.

**Dental Health, Stature, Socioeconomic Status**

## H21  Past or Present? An Empirical Basis for Quantitatively Distinguishing Between Prehistoric and Modern Forensic Cases Using a California Native American Population

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The goals of this presentation are to discuss the need for a *Daubert* standard method for assessing the forensic significance of skeletal remains, and to determine the discriminatory statistical power of metric variations among prehistoric California Native American remains, indigenous Central American skeletal populations, and skeletal populations common in the forensic and archaeological context.

This presentation will impact the forensic community by explaining the general lack of qualitative and quantitative data available to identify the range of Native American populations aside from indirect expertise gained from case work. This clearly limits the confidence/ability of the forensic anthropologist in correctly allocating possible Native American remains to the forensic or prehistoric context. Metric and non-metric analyses are needed to distinguish between Native American populations and any potentially confusing cases of modern indigenous populations that may exhibit similar characteristics such as tooth wear, Inca bones, etc. The continuing influx of indigenous immigrants into California and other U.S. areas warrants the attention and refinement of forensic anthropological methods.

The goal of this presentation is to provide new data and statistical analyses for differentiating between prehistoric Native American remains and modern forensic remains. Presently, forensic anthropological standards for identifying Native American remains are based on metric and non-metric data collected on prehistoric and contemporary Native American populations from the American Southwest (see Rhine 1990, Ousley and Jantz 2005). While quantitative data are accessible for these populations, forensic criteria used to identify prehistoric Native American remains are largely dependent on field assessments of contextual information such as body position, grave goods, tooth wear, bone color, and bone texture/quality. However, as the postmortem interval widens, it becomes more difficult to apply these contextual criteria to determine antiquity, and the forensic anthropologist must rely on skeletal characteristics to determine if the remains are firstly Native American, and secondly if they are of prehistoric or forensic context.

The University of California at Santa Cruz forensic anthropological team has collected metric data on prehistoric California Native American remains. The data were analyzed to be integrated into current forensic anthropology statistical methodology for estimating the forensic significance of skeletal remains that apply to this geographical region of casework. From this presentation, attendees will better understand of the discriminatory statistical power between Prehistoric California Native American remains, indigenous Central American skeletal populations and skeletal populations common in the forensic and archaeological context.

**Forensic Anthropology, Native American, Metric Analysis**

## H22  Frequencies of Non-Metric Characteristics in Northern California Native Populations: Establishing a Foundation for Comparison

*Cris E. Hughes, MA\*, and Chelsey Juarez, MA, Department of Anthropology, University of California – Santa Cruz, Social Science 1, 1156 High Street Room 435, Santa Cruz, CA 95064*

After attending this presentation, attendees will gain a clear understanding of the frequencies of commonly utilized non-metric traits present in northern California Native Americans, and how these frequencies compare to published literature on other Native American populations and modern Latino populations.

This presentation will impact the forensic community by initiating the creation of new non-metric standards on large samples of modern and non-modern populations.

The goal of this presentation is to present a comparison of frequencies for commonly utilized non-metric traits in Northern California Native Americans, United States Latinos, and indigenous Guatemalans.

As forensic scientists we produce legal documents, the foundations of which must be based on empirical studies. Non-metric ancestry data are often included in these reports; however, these data are based on inadequate samples either due to sample size or population representation. Despite the fact that non-metric assessments are still common place in forensic case reports on Latino groups there is little basis for these assessments in the published literature. In effect, non-metric analyses conducted on these remains are based on frequencies founded on potentially non-similar populations.

Recently, the frequencies and utility of non-metric features associated with specific races/populations have been challenged (Hefner, AAFS abstract 2007). These studies suggest that forensic anthropologists cannot rely on older studies to provide accurate information on frequencies for certain populations such as Latinos and that the overall utility of non-metric data must be further investigated. This work demonstrates the need to make new standards on large samples of modern populations and to simultaneously investigate the utility of non-metric analysis. The research discussed in this presentation will contribute to this goal.

Members of each mentioned group were analyzed for 33 common non-metric traits as established by Rhine (1990). Two observers made all observations and were tested periodically throughout the analysis for inter-observer error in trait assignment. The Native Northern Californians represent both a spatial and temporal analysis of non-metric characteristics for the region. The United States Latino and Indigenous Guatemalan populations represent a modern forensic context. The

collected data was analyzed to develop an empirical, quantitative methodology for estimating the forensic significance of non-metric traits within these Asian stem groups and then compare them to previously reported frequencies.

From this presentation, the audience will take away a greater understanding of the empirical utility of non-metric analysis of traits in Prehistoric California Native American remains, indigenous Central American skeletal populations, and modern U.S. populations.

**Non-Metrics, Ancestry, Standards**

## H23 A New Metric Procedure for the Estimation of Sex and Ancestry From the Human Innominate

*Alexandra R. Klales, BA\*, Jennifer M. Vollner, BS\*, and Stephen D. Ousley, PhD, Mercyhurst College, Department of Anthropology & Applied Forensic Science Program, 501 East 38th Street, Erie, PA 16546*

After attending this presentation, attendees will learn of a new metric approach to estimate sex and ancestry of the human innominate that utilizes both easily repeatable measurements and well defined landmarks. Attendees will also learn of the benefits of using a Microscribe digitizer to calculate the aforementioned measurements and for data collection.

Sex and ancestry estimation are essential for the assessment of biological profile in both forensic cases and in bioarchaeological analysis. In turn these estimations are necessary to asses other aspects of biological profile such as stature and age. This presentation will impact the forensic community by demonstrating an approach that combines both old and new metric sex and ancestry estimation techniques of the human innominate which are both more reliable and more accurate than previously published methods.

The human innominate has previously been examined through multiple metric and non-metric studies and, based on morphological characteristics, has been determined by many to be the most accurate bone for sex estimation. However, in light of *Daubert vs. Merrell Dow Pharmaceuticals, Inc.* (1993), most previous methods fail to meet the *Daubert* requirements or are of questionable reliability and validity; therefore, an opportunity to improve upon past research is presented. In addition, an attempt was made to examine the usefulness of these measurements for ancestry estimation.

A sample of 77 left innominates from the Hamann-Todd Collection, housed at the Cleveland Museum of Natural History, was utilized in this study. All individuals were adults, at least 19 years of age, of known sex and ancestry. A total of 22 measurements were taken by two observers, first as a preliminary study using sliding calipers, and then at a later date using a Microscribe G2 Digitizer to acquire 21 landmarks representing the previous 22 measurements. All data was then entered into Fordisc 3.0 (Jantz & Ousley, 2005) and analyzed through discriminant function analysis (DFA) with forward stepwise selection for sex, ancestry, and for joint sex-ancestry estimation.

This study used a sample of individuals of documented sex, ancestry, and age to achieve the following goals: (1) to qualify previously studied non-metric sex estimation traits through metric analyses, (2) to test previously defined metric methods for reliability and validity, (3) to refine previously published measurements by developing new landmark definitions that are easily understood and landmarks that are easily identifiable and produce reliable measurements, (4) to analyze the significance and utility of the new measurements through discriminant function analysis, and (5) to compare the concordance of measurements taken with calipers to those taken with a Microscribe digitizer. Furthermore, this study investigates the effectiveness of these measurements in ancestry estimation and also in joint sex-ancestry estimation.

Initial results indicate that measurements from the current study show greater reliability and validity with the use of discriminant function analysis than previously published measurements, while also producing a known error rate in accordance with the *Daubert* requirements. Benefits of using the digitizer include: increased data recording efficiency, fewer data entry errors, and the localization of landmarks in 3-D space. The current study will positively benefit future sex estimation using the innominate and will demonstrate the advantages of digitizing the innominate over the use of sliding calipers, though either can be used for sex estimation using DFA. In addition to sex estimation, the current study will also discuss the effectiveness of using the innominate to estimate ancestry and simultaneous sex and ancestry.

**Innominate, Sex and Ancestry Estimation, Discriminant Function Analysis**

## H24 Secular Trends in Cranial Morphological Sexing

*Kanya Godde, MA\*, and Angela M. Dautartas, BS, University of Tennessee, 250 South Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will understand the changes in cranial morphological sex characteristics between individuals with birth years from the 1840s through the 1960s.

This presentation will impact the forensic community by extending an anthropologist's ability to properly assess sex in an unidentified individual. Awareness of the changes over time in skeletal morphology will strengthen skills for accurate construction of the biological profile.

Hrdlicka (1920) originally created the now popular cranial morphological sexing criteria based on his reading of various German and French papers. He did not standardize his method; rather, he mostly described the morphological features with size terms, such as "medium," and no sketches (Hrdlicka 1920: 91). In 1994, Buikstra and Ubelaker published sketches and descriptions of each morphological characteristic in order to standardize the method. Other than Buikstra and Ubelaker's (1994) contribution, 74 years after Hrdlicka's descriptions, the method has not been officially outlined.

Despite its lack of formal definition until 1994, the technique was used in anthropology since Hrdlicka's (1920) adoption of the methodology. Application of the technique has been taught with the caveat of needing to understand the variations in size and morphology in the population with which it is utilized. However, there has been little research on how cranial characteristics in the American population have changed over the last century and thus there is little insight into American morphological variation over time. Since the method was originally adopted in 1920, the American population has changed greatly due to proper nutrition and better healthcare, among other factors. These changes include increases in average weight, stature, and overall health. In order to properly employ the cranial morphological sexing technique in a forensic setting, anthropologists need to be aware of potential changes in morphology over time. This knowledge will help with assessing sex in forensic cases where bones are discovered decades after the individual's demise, as well as with contemporary cases. Thus, it is hypothesized that secular trends have also occurred in cranial morphology in Americans, which affects the utilization of the sexing technique.

In order to test the hypothesis, 516 male and female American Blacks and Whites from two skeletal collections (the Hamann-Todd Collection and the William M. Bass Donated Skeletal Collection) were observed for each of the five cranial morphological sexing characteristics. These traits include: mental eminence, supraorbital margin, supraorbital ridge, nuchal crest, and mastoid process. Additionally, Howell's mastoid measurements (mastoid length and mastoid breadth), and a new measurement, mastoid width, were

collected in order to calculate the volume of mastoids. Mastoids resemble cones more than any other geometric shape, and thus the three measurements collected in this analysis were selected and designed to simulate this form. The birth years from these collections span more than a century; they range from the 1840s to the 1960s. The data were split into two samples based on sex (Cridlin et al. 2008), and each of these two samples was independently analyzed.

A time series statistical analysis was executed on transformed categorical and untransformed continuous variables for both sexes. The results indicate that over time the variables exhibited either a positive or negative trend. Most notably, supraorbital ridge increased over the last century.

The results here suggest that the secular trends in American Whites and Blacks warrant modifying the application of the technique to accommodate change over time in the American population. Recognition of these changes will strengthen the methodology behind building a biological profile. While these results reflect secular trends in the American population, they are not meant to be extrapolated to other populations. Each population has its own respective history and changes in skeletal morphology reflect events and trends within it.

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**Cranial Sexing, Secular Trends, Cranial Morphology**

## H25 Determination of Sex Using Metric Data of Greater Sciatic Notch in Koreans

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The goal of this presentation is to suggest that the greater sciatic notch could be helpful for sex determination in forensic anthropology. The metric data of the greater sciatic notch are highly dimorphic between Korean males and females and a discriminant function analysis was found to classify sex with high accuracy.

This presentation will impact the forensic community by explaining how it is straightforward to measure the width and depth of the greater sciatic notch. Moreover, metric analysis for the determination of sex from the greater sciatic notch was found to be highly accurate. These results are helpful in the determination of sex in Korean skeletal remains by utilizing more objectivity than non-metric traits for the greater sciatic

notch.

The hip bone is very helpful in the determination of sex of unknown human remains. In many cases the morphology of the greater sciatic notch (GSN) is used for sex determination through non-metric observation according to five grades. Non-metric observation is good for obtaining an immediate result; however, the result strongly depends on the experience of the observer and it is sometimes inaccurate. Recent study has shown that the non-metric method has the potential for conversion into a metric method, which provides objectivity for forensic anthropological analyses. To this end, the morphology of the GSN was investigated metrically in order to determine sex by numeric standards.

From the human bone collection of Yonsei University College of Medicine, 164 GSNs with known sex and age information at death were measured using digital calipers. GSNs were obtained from 112 male cadavers and the remainder were from females. Width and depth of the GSN were measured and the depth-width index of the GSN was calculated. The angle of the GSN was computed using measurements and a trigonometric function. The accuracy of sex determination was analyzed statistically by discriminant function analysis in SPSS (version 13, IL, USA).

Metric data of the GSN are highly dimorphic between Korean males and females. Width of the GSN in males was narrower than in females. Depth of the GSN in males was longer than in females. The accuracy was 84.6% for the depth-width index of the GSN and 89.5% for the calculated angle of the GSN. The demarking point was 65° for the calculated angle of the GSN and 68 for the depth-width index.

It is straightforward to measure the width and depth of the GSN. Moreover, metric analysis for sex determination from the GSN was found to be highly accurate. These findings are useful in the determination of sex from the GSN of Koreans, and the results provide more objectivity than with non-metric trait methods.

**Greater Sciatic Notch, Sex Determination, Korean**

## H26 Sexual Dimorphism of Joint Surface Area through 3-D Digital Data Modeling

*Denise To, MA\*, JPAC-CIL, 310 Worchester Avenue, Building 45, Hickam AFB, HI 96853*

After attending this presentation, attendees will gain insight into the sexual dimorphism of the surface area of four skeletal articular elements, learn of the feasibility of using this method to determine sex of an unknown skeleton, and gain a better understanding of the research potentials of 3-D modeling and digital data acquisition.

This presentation will impact the forensic community by elucidating the variation of an easily observed variable that is rarely used in osteological research and by fostering interdisciplinary research approaches in physical anthropology by incorporating 3-D technologies.

The surface area of skeletal features is easily observed but because its quantification is difficult to capture by traditional approaches, it is rarely included in osteological research. As a result, variation in the surface area of human skeletal features has never been thoroughly investigated. This study investigated the relationship of joint surface area with sex by applying three-dimensional modeling and digital data collection techniques normally used in engineering and computer science applications. In physical anthropology, research designs that have employed 3-D techniques (mostly in the analyses of primates and fossils) are limited. In addition, many studies that have included observations of surface area have quantified it through indirect expressions. Here, laser-assisted stereo modeling was utilized to reverse engineer skeletal features that allowed for accurate and reliable digital quantification of joint surface area.

A total of 810 virtual models of joints were stereologically created with an optical laser scanner from 211 adult skeletons drawn from the Robert J. Terry Anatomical Collection. Four surfaces from three joints

(femoral head, humeral head, glenoid fossa of the scapula, and auricular surface of the ilium) from each skeleton were modeled with a Cyberware Inc., Model 15 scanner. A watershed-based segmentation software was used to isolate the articular surface of each joint, and a region editor program was used to measure the isolated joint surface.

All four surfaces were found to be sexually dimorphic. Univariate discriminant function analyses on each variable produced cross-validation accuracies between 69.4% using the auricular surface area and 87.2% using the humeral head area. Only the area of the humeral head and the glenoid fossa were selected for determining sex with a multivariate linear discriminant function analysis. Correct classification using this function was found to be 90.0%. Surface area was found to be a better indicator of size and sexual dimorphism than linear long bone dimensions.

This project has significant ramifications. First, its focus on surface area addressed research questions of an easily observable, yet largely ignored biological feature. The high accuracy percentage found in determining sex with a multivariate discriminant function with the area of the humeral head and glenoid fossa indicates that these are highly useful criteria. In contrast, the exclusion of the area of the femoral head from the function suggests that perhaps further research into its human variation is warranted given its highly-used linear variables in determining sex. Second, this project made effective use, on a large scale, of a relatively new method of 3-D digital data acquisition that has only begun to demonstrate its vast applications. While time consuming, the virtual methods used here may serve as a springboard for other studies to incorporate 3-D quantification of surface area into their research designs. This technology presents physical anthropologists with much-needed innovation to conduct research. Finally, 3-D digital modeling can produce such an accurate virtual model of a bone that a quasi-permanent replica can be made available to students and researchers instead of the actual specimen. The ramifications are substantial as 3-D digital modeling can create a virtual library for future generations that can help preserve priceless skeletal collections, such as the Terry Collection. While a virtual model may not always be a perfect replacement for the real thing, this research project demonstrates that, in some cases, a virtual model is arguably *better* than the real thing.

**Surface Area, Sexual Dimorphism, 3-D Imaging**

## H27  Sex-Determination of Koreans Using Metric Analysis of Vertebrae

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After attending this presentation, attendees will understand the results of sex determination of Koreans using metric analysis of vertebrae from the documented skeletal collection housed at Yonsei University in Korea.

This presentation will impact the forensic community by presenting the results of metric analysis about the cervical, thoracic and lumbar vertebrae, and the usefulness of the documented skeletal collection housed at Yonsei University for determination of the biological profile in Koreans.

Physical anthropology is a branch of anthropology concerned with the study of human evolution and human biological variation, including the investigation of the morphological characteristics of the human body including bone. There are two main ways to collect anthropological data: metric and non-metric. Through this process, physical anthropologists interpret and understand the characteristics of a population, such as biological evolution and genetic inheritance. In order to appreciate the characteristics of a population, it is important to statistically study documented human skeletal specimens. On the basis of these results, a reconstruction of the biological profile for unidentified skeletal remains can be possible. The Department of Anatomy, Yonsei University College of Medicine in Korea has collected skeletons after anatomy class since the 1990's, and about 100 specimens have been collected. This study will present the results derived from the documented skeletal collection at Yonsei University, which pertains to sex determination in Koreans based on metric study of vertebrae.

For this study, 34 whole vertebral columns were selected out of the total Yonsei sample since many cervical vertebrae were missing from the anatomy class and skeletal preparation. These consisted of 22 males and 12 females, and the average age was 54 years (from 18 to 88 years). For the thoracic and lumbar vertebrae there were a total of 9 measurements: upper & lower end-plate width, depth of upper end-plate and lower end-plate, anterior height and posterior height of vertebral body, transverse diameter of vertebral foramen, sagittal diameter of vertebral foramen, and width of vertebra. In the cervical region, measurements were taken of the length of the spinous process instead of the depth of the upper end-plate and lower end-plate because the vertebral body of the cervical vertebrae have the uncinate process. Measurements for atypical vertebrae (i.e., the atlas and axis) consist of 23 and 13 measurements, respectively. All measurements were taken with a digital caliper (Mitutoyo Co., Japan) and statistical analysis was performed using SPSS (version 13.0).

The measurement values related with the vertebral body dimensions, such as upper & lower end-plate width, were highly dimorphic between males and females. Also, results show that the measurement values were related to the size of the vertebrae, such as width of vertebra. The discriminant function equations were obtained by univariate, bivariate, and step-wise methods. The range of accuracy of the cervical vertebrae was from 78.8 to 100%, and accuracy for the thoracic vertebrae was from 86.1 to 100 %, and for the lumbar vertebrae it was from 76.5 to 97.1%. The accuracy of the 5th cervical vertebra, 3rd and 11th thoracic vertebrae, and 1st lumbar vertebra showed the highest accuracy of sex classification.

This presentation could indicate that metric data of vertebrae are useful for sex determination in Koreans, especially in the vertebral region where movement is frequent. However, further investigation is necessary to increase the sample size and work will continue in order to study the vertebrae from the digital Korean human model established by Computer Tomography (CT). Finally, the documented skeletal collection at Yonsei University is a useful resource for research related to the Korean biological profile.

**Koreans, Sex-Determination, Vertebrae**

## H28  Tarsal Measurements to Estimate Sex for Use in a Forensic Setting

*Vanessa L. Aziz, BA\*, 11735 Bergamo Court, Las Vegas, NV 89183*

After attending this presentation, attendees will learn that osteometric analysis of the tarsals can produce accurate determination of the sex of the decedent in forensic cases. While previous studies in the

past have utilized the calcaneus and talus for osteometric analysis to determine sex in both modern and prehistoric populations, this study has utilized all seven and found that higher levels of accuracy are achievable using all seven tarsals in the sample population studied. Attendees will also learn how to accurately measure all seven tarsals to reproduce the results of this study.

This presentation will impact the forensic community by providing new ways of determining the sex of the decedent. This study contributes to the forensic community, and specifically the forensic anthropology community, as the identification of an individual based upon skeletal traits is of primary concern. This study does just that by helping to predict with a certain amount of accuracy the sex of an individual based upon the tarsals alone.

Identification of an individual based on skeletal traits is of primary concern in forensic anthropology. A basic antemortem profile should include age, ancestry, and sex; factors that can help law enforcement identify the decedent (Bass 2005). Although there has been extensive research and testing of many bones as indicators of age and sex, often these bones are not available in a forensic setting. More research is needed on those bones which can withstand greater extremes such as weathering and fire, since these elements are often those encountered in forensic settings. The irregular bones of the ankle fit such criteria, as they are relatively dense in some areas (White 2000). While several studies have been done using the calcaneus and talus, (Gualdi-Russo 2007, Steele 1976, Murphy 2002, 2005) no significant contribution to the study of modern populations have been published concerning all seven tarsals (Bass 2005; Bidmos and Asala 2003, 2004; Gualdi-Russo 2007; Murphy 2002a, 2002b; 2005; Steele 1976; Wilbur 1998). This current project advances these previous studies by publishing data and analysis of tarsal dimension patterns for a museum population of individuals who died within the last 25 years and are curated at the University of New Mexico.

Several sources have studied the measurements of the calcaneus and talus in relation to sex on prehistoric populations. Murphy (2002a, 2002b, 2005) studied the talus and calcaneus of prehistoric New Zealand Polynesians. Wilbur (1998) published on the subject of hand and food bones for the determination of sex in a prehistoric population from West-Central Illinois.

Modern population specific research has been done on the calcaneus and talus as well. Using five measurements each from the talus and the calcaneus on a modern American ancestral white and black population, Steele (1976) accurately predicted sex 79% to 89% using a discriminant function analysis. Gualdi-Russo (2007) has produced a high percentage of correct classification of sex (87.9-95.7%) within a modern northern Italian population. The tali and calcanei from the Raymond A. Dart Collection of Human Skeletons were utilized in two studies by Bidmos and Asala (2003, 2004) to determine sex in South African black and whites and Bidmos and Dayal (2004) applied a discriminant function analysis to the tali of a South African white population, yielding 80% to 82% accuracy for the univariate method, 85%-88% for the stepwise method, and 81% to 86% for the direct method.

The purpose of this study was to see if sexual dimorphism in all seven tarsals among a modern North American documented skeletal collection was statistically viable, and if so, how this might aid the forensic community. In this current study, all seven tarsals from a modern North American population were measured – the calcaneus, talus, navicular, 1st - 3rd cuneiforms, and the cuboid – to determine if there is a clear discrimination between the sexes. These measurements were subjected to univariate and stepwise discriminant function analysis to determine the percentage of accuracy achievable.

Utilizing a selection of 69 adult male and female individuals ranging in age from 18 to 101 from the modern documented skeletal collection housed within the Maxwell Museum at the University of New Mexico in Albuquerque, NM, this study has found that an accurate sex assessment of 94.24% can be achieved utilizing measurements from all seven tarsals, and that single measurements can predict sex up to 88.41% accurately.

**Tarsals, Sex Determination, Osteometric Analysis**

## H29 An Evaluation of Facial Features Used for Facial Recognition Applied to Cases of Missing Persons

*Samantha M. Seasons, BA\*, and Erin H. Kimmerle, PhD, University of South Florida, Department of Anthropology, 4202 East Fowler Avenue, Soc 107, Tampa, FL 33820*

The goals of this study are to demonstrate a simple method for creating 2-D composites for facial reconstructions and to evaluate the accuracy of this method through both qualitative and quantitative methods. This research identifies what facial features are most recognizable and key components for successful recognition.

This presentation will impact the forensic community by explaining several important outcomes that are noted from the study. First, 2-D composites are shown to be accurate with a high rate of recognition among people who did not know the victim. It also highlights which facial features are most important in facial recognition and the differences among males and females in how they recognize faces. One critical outcome of this research is that it demonstrates that the quality and intrinsic properties of the reference photograph given by families to law enforcement may also play a role in recognition among cases of missing persons. Therefore, the image obtained by police in missing persons is as important as accuracy in facial reconstructions created for unidentified decedents. The importance of accurate and time efficient methods for facial reconstructions, which are based on an understanding of how specific facial features impact facial recognition and the variation among observers, may aid law enforcement agencies and increase the number of identifications for missing and deceased persons.

The problem of missing persons and unidentified decedents, specifically how to link individuals within these two arenas, is critically important for families and for judicial accountability in cases of homicide. Due to the increased demand to identify missing persons globally, the role of forensic anthropologists in those types of investigations is expanding. Forensic anthropologists construct a biological profile including the sex, ancestry and the approximate age of the decedent, which provide the foundation for facial reconstructions. Facial tissue depth data along with the biological profile are used to create composite images using computer software programs such as Photoshop CS and FreeFormÒ Modeling Plus.™ Recent advancements in 3-D imaging have resulted in virtual applications of facial reconstructions. Such technologies, however, may not always be available to all investigators particularly in human rights investigations.

The purpose of this study is to: (1) evaluate the accuracy of craniofacial reconstructions created through 2D composites, (2) identify what facial features are most recognizable, and (3) identify patterns among respondents that may influence recognition such as gender, age, or the quality of the reference (missing person) photograph. For this study, four composite images were created for three skulls, using photo superimposition techniques. A total of 120 students from the University of South Florida were asked to match the composite image with the picture of who he most looked like (out of six possible choices). Among the comparison images, one image was the actual decedent. Participants were randomly surveyed about the likeness of the images and the ranked order of likeness. Demographic data about the participants was also collected. Qualitative descriptions about similarities and differences

were collected from each participant. Further, *Pearson's Chi Square* tests were used to test the significance of the likeness among the composite and photographs and the responses from the survey.

In two of the four cases, the composite image and the actual decedent photograph were said to be *very similar*. In one case, the same composite image was used but the missing person image was changed. In this case, it was said to be *not at all similar*. This highlights an important point - that recognition is not only important on the level of the created composite but also in the intrinsic factors of the missing person photograph (i.e., the presence of a hat, certain clothing, the angle of the face, background, or shading contrast). It is shown that by changing the picture of the missing person that is used to elicit recognition, the outcome varies. Therefore, the picture families give to law enforcement in missing persons cases may affect the how likely someone is to recognize that person.

Other patterns are also discussed, such as differences among males and females in recognizing the same faces. For example, out of 52 male participants and 68 female participants, 25% (13/52) of males ranked a composite photograph as the most similar whereas in the same case, only 7.4% (5/68) of the females ranked it as the most similar. Among traits that are used to evaluate the likeness of two images, the nose was the most common trait used according to 76.5% of participants, followed by the eyes. Interestingly, the nose and eyes are largely subjective areas in creating composite images since the particular morphology and color can not be estimated from skeletal remains. Yet, participants who said those were the most important traits still correctly matched the composite images with the actual photograph of the deceased. It is evident that the relationship between different facial features and the overall composition of the face does influence the rate of facial recognition.

**Facial Reconstruction, Recognition, Missing Persons**

# H30  The Reliability of Visually Comparing Small Frontal Sinuses

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The goal of this presentation is to provide attendees with information about the reliability and validity of visually comparing and correctly matching small, less-featured frontal sinus outlines as seen in radiographs.

This presentation will impact the forensic community by further emphasizing the reliability of personal identification through matching frontal sinus radiographs.

Several studies have investigated frontal sinus comparison for personal identification. Many of these studies, however, involved small sample sizes both in terms of the number of radiographs examined and the number of participants. One study addressed the statistical reliability of correct identification using Elliptic Fourier Analysis and Euclidean distance models on digitized images which resulted in a 96% accuracy rate. The remaining 4% largely represents the inability of the computerized models to correctly match small, less-featured frontal sinuses.[1] The present study investigates the hypothesis that human examiners will be able to more accurately identify correct matches both because of the discriminating ability of the human eye as well as the potential to take other features of the radiograph image into consideration.

Radiographs were obtained from the University of Tennessee and represent specimens from the William M. Bass Donated Skeletal Collection taken as part of a previous study. A random sample of 60 pairs of radiographs was selected from the collection. From these 60, the radiographs with the smallest frontal sinuses and lacking visible dental restorations were used for this study, thus creating a sample specifically aimed at making the matching process as difficult as possible.

Participants of varying backgrounds and levels of experience were solicited to participate in the study including Federal Bureau of Investigation scientists and attendees of the 2008 annual meeting of the American Academy of Forensic Sciences in Washington, D.C. Participants were provided two sets of 28 radiographs labeled A through BB and 1 through 28, an answer sheet, and a light box. They were advised that matches consisted of one letter or letter combination plus one number, and that not all radiographs necessarily had a corresponding match present.

Participants were also asked to provide information regarding their education and background. Further, they were asked to rate their level of experience in both examining radiographs and performing anthropological or skeletal examinations. Finally, participants were asked to list any characteristics besides the frontal sinuses that they used to determine matches.

The exercise contained 26 matched pairs and four radiographs that did not have a match. Overall, error rates were very low. False negative associations were significantly more common than false positive associations and errors generally occurred less frequently among participants with more experience. Of note is the fact that one particular association appeared to be the most difficult to identify and was missed most frequently. Most participants reported using features in the radiographs in addition to the frontal sinuses to make identifications.

Results support previous assertions that frontal sinus radiographs are a reliable means of personal identification. Moreover, while previous studies have statistically evaluated the technique's reliability using computerized models, the results of this study indicate that traditional visual comparison fares exceptionally well, even when frontal sinus projections are small.

**Reference:**
1    Christensen AM. Testing the reliability of frontal sinus outlines in personal identification. J Forensic Sci 2005;50(1):18-22.
**Forensic Anthropology, Frontal Sinus, Personal Identification**

# H31  Three-Dimensional Computer Modeling and Anthropological Assessment of the National Library of Medicine's Visible Human Male

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The goal of this presentation is to demonstrate the ability to establish a biological profile used by forensic anthropologists in human identification from three-dimensional (3-D) image data. Using previously validated methodologies,[1] it will also compare the computed models of the cranial and post-cranial skeleton to those printed by a 3-D rapid prototyping machine.

This presentation will impact the forensic community by increasing scientific knowledge of new methods available for use in training and the identification of human remains.

Using three-dimensional (3D) imaging technology, researchers have been able to create virtual computed models of anatomical structures for a wide range of educational and research activities. The purpose of this study was to demonstrate the potential for establishing an

accurate biological profile of a human skeleton solely from reconstructed 3-D data of the National Library of Medicine's *Visible Human Project®.*[2]

This study began by utilizing the Visible Human Male's (VHM) anatomic serial sections to isolate the skeletal tissue from each slice. Every anatomical structure in the digital image set was assigned a unique color and isolated using Adobe *Photoshop CS3©*. These image files (.tiff) were then imported into the software package, Mimics© version 12 (Materialise) for reconstruction and 3-D visualization.

After the complete skeleton of the VHM was reconstructed, 78 anthropometric cranial and post-cranial measurements were selected from an index of standard anatomical landmarks based on their effectiveness in establishing a biological profile in skeletal analysis.[3] Additionally, non-metric traits commonly used in the assessment of age, sex and ancestry were also examined to complete the biological profile. Once the anthropological data was collected, the biological profile was then compared to the known values of the subject documented by Spitzer, et al.[2]

The 3-D virtual models of the skeleton were exported as stereolithographic (STL) files and a select series of bones were printed using a Zcorp 3-D ZPrinter© 310 Plus rapid prototype machine. The prototype bones were then measured with traditional caliper methods using the same indices as the virtual skeleton and compared to the previous results.

In this study, the biological profile generated from the virtual skeleton was consistent with the known information about the Visible Human Male. Statistical analysis of the data comprising the samples (virtual and prototype), confirmed the accuracy of the computer modeling and measurement technologies. Additionally, this study found the prototype bones to be valuable reproductions, even taking into consideration artifacts from the printing process.

This study demonstrates that 3-D datasets of different kinds (digital images and serial sectioning) can be useful tools in the study of human anatomy for clinical, educational, and forensic purposes. Data sets that are public domain such as the National Library of Medicine's *Visible Human Project®* have proven to expand the accessibility of anatomical specimens beyond actual contact. The data resulting from the *Visible Human Project®* has allowed for the possibility of creating reliable virtual models for teaching purposes as well as models for testing fundamental anthropological methods, like establishing a biological profile, through the use of 3-D volumetric data reconstruction.

**References:**

1.  Decker SJ, Hilbelink DR, Hoegstrom EJ. Virtual skull anatomy: three-dimensional computer modeling and measurement of human cranial anatomy. Proceedings for the 60th Annual AAFS Meeting, Washington, DC 18-23 Feb. 2008; (14)312.
2.  Spitzer V, Ackerman MJ, Scherzinger AL, Witlock D. The Visible Human Male: A Technical Report. *Journal of the American Medical Informatics Association*, 1996; 3:118-130.
3.  Buikstra JE, Ubelaker DH (eds.) (1994) *Standards for Data Collection from Human Skeletal Remains.* Arkansas Archaeological Survey Research Series No. 44.

**3-D Imaging, Computer Modeling, Human Anatomy**

## H32 The Reproducibility of Results From Facial Approximation Accuracy Tests That Use Face-Arrays

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After attending this presentation, attendees will gain insights into the differences that can be expected when accuracy tests of facial approximations are conducted using different groups of assessors but the same face-array test.

This presentation will impact the forensic community by demonstrating the reliability of the most favored method for assessing facial approximation performance.

The use of facial approximation methods in the forensic arena makes knowledge of these methods accuracy and reliability pertinent. In the recent scientific literature it is now common for the face-array test to be advocated as the most favored form of performance assessment. If, however, the results from face-arrays are to be confidently interpreted then their reproducibility must be known. So far, repeatability studies using the same facial approximations but different assessor groups have not been performed. Clearly, more reliable results are expected with larger samples, but what numbers of assessors are needed and how reliable the results are for any sized assessor sample is currently unknown.

This study investigates the repeatability of face-array results for three previously published facial approximations by employing three new groups of assessors in addition to the original study's sample. In each case, face-arrays consisted of ten faces (including the target individual) with retest assessor sample sizes approximating 40, 75, and 115 individuals. The results show that fluctuations in the recognition rates up to 20% were not uncommon for single faces between samples, even when large assessor groups were employed (75-115 assessors). These data thereby suggest that recognition rates for single faces should only be considered to be approximate and that heavy weight should not be assigned to isolated recognition rates for single faces. If marked peaks are present in the recognition profile of the array, reliable indications to a facial approximation's accuracy may be gleaned from even small sampled studies, however, if such peaks are absent repeated tests using different assessor groups or a single large assessor samples (*at least* >75 individuals) are advisable. Since it appears that fluctuations in recognition rates between groups are not dramatically reduced with 75 to 114 assessors, it seems that much larger sample sizes might be needed to generate more reproducible results (but how large these samples must be is currently unknown). Future research must address this issue. Also, since recognition rates generated from face-arrays are expected to be heavily influenced by which foil faces are selected for inclusion into the array (in addition to the quality of the facial approximation), future research should examine methods for measuring and optimizing photo-spread fairness.

While face-array recognition tests are time intensive to conduct, the results of this study demonstrate value for obtaining larger assessor samples to run repeat face-array tests with multiple (independent) assessor groups. Repeat tests might be superfluous if very large numbers of assessors are used in any one pass at a face-array, but the exact number of assessors required to generate such robust results is currently unknown and could be unexpectedly large.

**Facial Approximation, Facial Reconstruction, Facial Reproduction**

## H33 Cranial Fracture Patterns in Pediatric "Crushing" Injuries and Preliminary Biomechanical Modeling Using a Simple Finite Element Model

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The goal of this presentation is to inform attendees about the characteristic fracture patterns that result from bilateral "crushing" (quasi-static loading) injuries of the subadult cranium by presenting several case studies of such insults and a simple biomechanical model that replicates and helps explain these patterns.

This presentation will impact the forensic community by demonstrating the type of fractures that can be expected in cases of bilateral crushing in subadults and attribute these characteristic patterns to the stresses developed in the cranium.

Bone fracture analysis is an integral component of some forensic casework. In terms of determining cause and manner of death, the forensic professional must possess a working knowledge of not only the forces involved in fracture production and propagation, but also the effects of geometry and stress concentrations. Most often blunt cranial injuries involve dynamic forces characterized by large energy changes generally due to rapid changes in velocity over the loading duration. Conversely, crushing injuries involve quasi-static forces that are applied slowly across broad aspects of the skull. The understanding of typical fracture patterns based on common scenarios and biomechanical modeling may be pertinent to the evaluation of cranial injuries sustained by subadults and attempts to determine circumstances of the injury (accident vs. abuse). If a fracture pattern is found which does not correspond to the adult's account, there may be need to inquire further to ascertain the true nature of the injury.

For this study, four cases are presented in which young children of various ages (from 1.5 to 6 years of age) have sustained fatal cranial crushing injuries from the tire of a slow moving motor vehicle or a heavy, non-motorized farm trailer. In each case the cranium was entrapped between the ground and the tire. In all four cases, the major bone fractures occurred in the basilar region of the cranium that bridges and links the loading sites (the tire and the ground) and traverses the middle cranial fossa in the area of the sella turcicum. Biomechanical engineering was then utilized in efforts to explain the mechanism of this fracture pattern using a mathematical model.

A model of a simplified cranium was constructed with symmetry about the sagittal plane, but not about the coronal or transverse planes. The base of the cranium was modeled as a flat surface with the same thickness as the dome and the primary landmarks in the basal region. A hole was added to simulate the foramen magnum, two dense spheres were placed anterior to the foramen magnum to represent the petrous portion, and the thickness in a section of the cranium between the petrous portions was hollowed out to represent the region of the spheno-occipital synchondrosis.

Finite Element Analysis (FEA) was conducted on the model by applying quasi-static, bilateral pressures. The pressures were applied to the model in such a way as to simulate the crushing forces reported in the four cases. The stress pattern developed in the simplified model revealed significant stresses on the base of the skull with areas of lower stresses on the cranial vault. The stress pattern corresponded well with the fracture pattern documented in the four cases. Further analysis revealed that tensile stresses were generated in the region of high stress that correlated with and explained the observed fracture pattern. This suggested that the quasi-static (slow) loading of the cranium leads to fracture in predictable ways.

The computational model suggests that the reason crania fracture through the basicranium is largely due to the concentration of tensile stresses due to the geometry of the cranium. Based on these pilot studies, it would appear that a simplified cranial model of a slow, "crushing" force could be used to help explain fracture patterns seen in typical case situations.

**Pediatric Fracture Patterns, Bilateral Crush Injuries, Finite Element Modeling (FEM)**

## H34 Shark-Inflicted Trauma on Human Skeletal Remains

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After attending this presentation, attendees will become familiar with shark-inflicted trauma on human skeletal remains. This type of trauma may be unfamiliar and/or not commonly seen by the forensic community.

This presentation will impact the forensic community by providing awareness and knowledge of a unique type of sharp trauma rarely encountered or observed by forensic anthropologists and other forensic scientists.

On August 16, 1997, the Jefferson Parish Coroner's Office was notified about the recovery of an unidentified male in a shrimper's trawl net in the Gulf of Mexico. At the time of recovery, the shrimp boat was 30 miles offshore at longitude 2829.9N and latitude 09026.3W. The U.S. Coast Guard retrieved the body from the shrimp boat and an autopsy was conducted at the Jefferson Parish Forensic Center on August 18, 1997.

According to the autopsy report, the body measured 5'11" in length and weighed approximately 83 pounds. The condition of the body was described as having "marked decompositional type changes present" with the head, right chest and back, left upper extremity, and both lower extremities being skeletonized. The right upper extremity was absent. "Irregular" gnaw-like marks were noted on the remaining tissue. Partially digested food was found in the stomach. The heart, lungs, liver, kidneys, spleen, esophagus, thyroid, pancreas, and adrenals were present. The brain was noted as being "generally liquid in nature." No definite signs of fractures, hemorrhage or other trauma, other than gnaw-like marks on the soft tissue, were identified. Based on the autopsy report, a postmortem interval of less than 48 hours from date of recovery was estimated.

On August 22, 2007, ten years after initial recovery, the unidentified remains, and associated clothing were picked up at the Jefferson Parish Forensic Center by the Louisiana State University Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory personnel and transported to the FACES Laboratory at LSU for analysis and inclusion in a cold case database. Upon arrival at the FACES Laboratory, the partially skeletonized remains were processed and cleaned.

Results of the forensic anthropology analysis revealed that the male decedent's ancestry was more consistent with black; however, due to some white and Amerindian characteristics, a Hispanic ancestry is possible. The decedent was 5'8" – 6'2" tall and between 30 – 45 years of age at the time of his death. Antemortem fractures were present on the nasal bones, the left frontomalare suture and the left zygomatic arch. The presence of bone remodeling and smooth edges indicated that all three fracture sites were in the process of healing prior to death and that

these fractures could have been the result of a single traumatic event for which the decedent may or may not have received medical treatment. The analysis also revealed that the decedent had an edentulous maxilla with pronounced bone resorption and remodeling, indicating that he had worn upper dentures for a long time. His mandible was toothless, except for the lower canine teeth, and vertical remodeling and thinning of the alveolar bone for all of the incisors were present. The decedent's lower canines, which were lost postmortem, had mechanically supported a partial lower denture by means of a "Dolder Bar" or similar oral reconstruction apparatus (Dr. Robert Barsley, Forensic Odontologist, personal communication).

Analysis of the postcranial elements revealed a remarkable and unusual series of individual incisions, overlapping striations, and punctures with or without associated fractures. The trauma was concentrated on the clavicles, right, and left ribs, on all three of the left arm bones, the left femoral head, the right hip bone, and along the entire shaft of the right femur. Based on the recovery location in the Gulf of Mexico, these types of trauma were probably caused by a combination of marine scavengers and predators. In fact, the overall pattern of the individual incisions, overlapping striations, and punctures, with or without associated fractures, are common bite mark artifacts produced by sharks (Dr. George Burgess, Director, Florida Program for Shark Research and International Shark Attack File, personal communication).

When a shark attacks, either as a scavenger or predator, water is displaced and pushed out in front of the shark like a wave. This shark-induced wave will cause the potential prey's body to move in the same direction as that wave. Just prior to their bite, sharks will close their eyes and rely solely on electromagnetic field sensing, which is not as accurate as sight orientation. The accuracy of the shark's bite is dependent on several variables. One variable is the water's natural wave motion. Another variable is the shark-induced wave, which is based on the size of the shark and its speed and direction of attack. A third variable is any movement(s) of the potential prey by its own volition or by either of the wave motions. Finally, the number of sharks and other marine life present will also affect the accuracy of a shark's bite. In deep water, the three most common shark species to attack humans are the White shark, the Tiger shark and the Bull shark. Bite trauma on the victim suggests at least one large, adult Bull shark and several other smaller species of sharks were involved (Dr. George Burgess, Director, Florida Program for Shark Research and International Shark Attack File, personal communication).

To assist with the identification of this individual, a clay facial reconstruction was completed. To illustrate both black and Hispanic ancestry, the clay facial reconstruction was then computer enhanced. The boots being worn by this male could also assist in his identification by way of a possible occupation. He was wearing steel-toed Tingley brand "Over-The-Sock Snugleg" boots. The manufacturer recommends this type of boot for the following applications: food processing, dairy, chemical, and petrochemical (exploration and production).

In the past, cases such as this had little opportunity for identification. With the current interest in creating and updating cold case databases across the country, the potential for positive identification of this victim is greatly enhanced. Finally, this presentation will provide forensic scientists with a better understanding of shark-inflicted trauma on human skeletal remains where case histories are sometimes unknown.

**Shark-Inflicted Trauma, Shark Attack, Human Skeletal Remains**

## H35 Patterns of Blunt Force Trauma Induced by Motorboat and Ferry Propellers as Illustrated by Three Known Cases From Rhode Island

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After attending this presentation, attendees will have a better understanding of the typical locations and injuries induced by motorboat and ferryboat propellers.

This presentation will impact the forensic community by illustrating three cases of propeller-associated fatalities. This information can be used as exemplar patterns of trauma when dealing with cases of remains recovered from marine contexts with unknown circumstances surrounding the death.

Understanding patterns of trauma is important when dealing with skeletonized remains as it may influence the determination of cause and manner of death. Further, an understanding of taphonomy and bone fracture mechanics is necessary in order to reconstruct the timing of injuries (peri- vs. postmortem) and establish or rule out fatal injuries. In cases of decomposed bodies recovered from marine environments, skeletal trauma analysis can be even more significant because of the multitude of confounding variables affecting the soft tissue. In these cases, it is the ability to recognize patterns of skeletal injuries and reconstruction of the taphonomic history of the individual that can be most helpful in understanding the circumstances of the death event.

Previously, Kroman *et al.* (2007) discussed experimental patterns of injury and injury mechanics from propellers in relation to speed at impact and propeller style. Their research discussed trauma in terms of blunt and sharp force trauma to human cadavers and euthanized pigs in an experimental setting. The present paper will attempt to further examine the skeletal trauma caused by similar mechanisms in terms of wound characteristics and location. With this information investigators may be able to identify propeller trauma, even in severely decomposed bodies.

In 2007, the Rhode Island Office of State Medical Examiners investigated three unrelated cases involving decedents who had been struck by boat propellers.

**Case 1:** A young male decedent was struck by the propeller of an open motorboat as it approached him at a reportedly low speed in the water.

**Case 2:** A young male decedent was struck by the propeller of an open motorboat traveling at a relatively high speed after falling overboard at the front of the boat.

**Case 3:** An elderly male decedent fell from a pier and was struck by the propeller of a ferry as it was leaving the dock.

The first two cases exhibit similar patterns with longitudinal parallel propeller blade impacts that traverse portions of the body. In each case the propeller impacted the cranium and the extremities. While impacts to the extremities and torso created skeletal trauma easily recognizable as blunt force, impacts to the cranium created somewhat linear fractures with primarily smooth fracture edges that could be mistaken for sharp force. However, they are considered the result of blunt impacts because the shape of the edge on a standard propeller blade is "squared-off," rather than beveled, which would result in sharp trauma. Due to the squared edges of the propeller blade, "scoring" can and does occur on the bone, however, this is not true sharp force trauma because of the lack of edge bevel. The decedent in Case 2 sustained

blunt force lacerations to the lungs and liver which appear as linear defects, yet are the result of tears in the soft tissue rather than incised cuts.

In Case 3, the impact from the ferry propeller caused significant damage to the decedent. In this case the body was completely severed in the abdominal region and only the upper portion was recovered. The torso showed fractures to every rib as well as both forearms; the left radius displayed a classic butterfly fracture. In this case, the blade of the propeller also impacted the cranium and caused delamination of the outer table, a characteristic of slow load blunt force trauma to the cranial vault. Vital organs were no longer present to evaluate at autopsy and cause of death was undetermined, however it was determined that the injuries caused by the ferry propeller were sustained postmortem.

These case studies review the traumatic blunt force characteristics caused by propeller injuries as well as highlight the anatomic regions most likely to sustain skeletal trauma. The first two cases exhibit blunt skeletal injuries caused by standard propellers at different speeds reflecting similar patterns of trauma. The third case sustained blunt trauma to the majority of the body due to the greater sized blade and energy associated with the large ferry propeller.

These case descriptions, coupled with an understanding of blunt force trauma biomechanics to bone, should allow the examiner to compare injuries, even in severely decomposed bodies, to patterns of documented propeller blade trauma as an aid to determining cause and manner of death in marine fatalities.

**Blunt Force Trauma, Propeller Injuries, Case Study**

## H36 Cervical Vertebrae Entrapment in the Noose as Evidence of Cause of Death by Hanging in Skeletal Cases: Three Remarkable Finds

*Jennifer A. Ledford, BS\*, Barrett Gobelet, BS\*, and Hugh E. Berryman, PhD, Department Sociology & Anthropology, Middle Tennessee State University, Box 89, Murfreesboro, TN 37132*

After attending this presentation, attendees will be shown examples of cervical vertebra entrapment in ligatures as evidence of cause of death by hanging.

This presentation will impact the forensic community by stressing the importance of thorough recoveries at skeletal crime scenes, especially in areas where bone and culturally pertinent associations are scattered among extraneous debris.

Cause of death by hanging may be difficult to determine from decomposed or skeletal remains alone, and may have to be inferred from evidence at the scene. The purpose of this paper is to present three remarkable skeletal cases, each exhibiting a cervical vertebra entrapped in a noose as unambiguous evidence of cause of death by hanging.

The first case involved human skeletal remains found in a wooded residential area in Middle Tennessee. Dr. Hugh Berryman, two members of the Forensic Anthropology Search and Recovery Team and a death investigator from the State Medical Examiner's Office processed the scene. The remains were clothed and largely held in articulation by dried soft tissue. No ropes or other indicators usually associated with hanging were found upon initial examination. Remnants of a dark colored woven belt tied to a wide strip of white cloth were found among other unassociated debris on the ground near the remains. When collected, it was discovered that the D-shaped metal buckle facilitated a loop that enclosed a skeletonized 5th cervical vertebra and hair. The metal buckle was positioned on the posterior side of the neck and the size of the loop was exceedingly small, indicating that it became reduced in size as the body decomposed.

A second case was discovered in eastern Louisiana in April 2006 and was presented at the 2008 annual meeting of the American Academy of Forensic Sciences by Dr. John Verano. The remains were recovered from a wooded area and were largely skeletonized, with some dried soft tissue, and clothed. The majority of the remains were found hanging in a partially fallen tree with the skull and other bones scattered in the area. In this case, a nylon rope with a simple slipknot was found to encircle the skeletonized 3rd cervical vertebrae and hair. The position of the knot is posterior and to the right of the spinous process, but due to decomposition, the knot may have slipped from its original position.

The third case from Pinellas, Florida dates to October 1990 and was examined by Drs. Douglas Owsley and Robert Mann. At the scene, police found human skeletal remains and what appeared to be men's clothing lying on the ground at the base of a tree from which a rope and noose still hung. A 4th cervical vertebra with a crushed right vertebral foramen was found encircled by the noose. Since this foramen was crushed, the position of the noose at the time of death could be determined and is said to indicate homicide as the manner of death.

In anthropological cases involving hanging where the bones and associated cultural items have been scattered, cause of death may be difficult to determine. This study presents three cases where the presence of a cervical vertebra entrapped in a noose provides clear evidence of hanging as the cause of death. Such findings argue for the thorough processing of skeletal crime scenes, especially in areas where bone and pertinent cultural associations are scattered among extraneous debris.

**Hanging, Cause of Death, Crime Scene Recovery**

## H37 Diagnosing Peri-Mortem Blunt Force Trauma in Burnt Remains

*Aimee E. Huard, MA\*, Binghamton University, Jeremy J. Wilson, MA, and Dawnie W. Steadman, PhD, Department of Anthropology, Binghamton University, PO Box 6000, Binghamton, NY 13902-6000*

The goal of this presentation is to utilize photographic and radiographic overlays enabling the participants to form their own opinion(s) regarding the etiology of observed fracture patterns (i.e., blunt force trauma vs. heat and fire-induced fractures).

This presentation will impact the forensic community by focusing on the combined effects of blunt force trauma plastic deformation and subsequent heat exposure on bone. Attendees will be provided with a basic model for reconstructing and differentiating fracture patterns.

Intentional clandestine burning is a relatively common manner of inhibiting discovery and identification of a murder victim. Forensic anthropologists may be called upon to distinguish between perimortem injuries and taphonomic modification (burning) of bone that confounds trauma interpretation. Controlled experiments are an ideal way to understand the fracture pattern of blunt trauma and burning. Previous themed experiments have focused on questions regarding bone shrinkage, alteration of bone histology, the relationship of discoloration to intensity of fire, and heat-induced fracture patterns. This experiment specifically explores gross radiographic and microscopic differences in peri-mortem blunt force trauma fractures as compared to those fractures that are the product of intense heat and fire.

This study focuses on the combined effects of plastic deformation of blunt force trauma and subsequent heat exposure on bone. Identification of plastic deformation in burned bone can be vital to understanding peri-mortem trauma, as the morphology of fractures indicates the forces involved and the direction of the mechanical loading. Damage to bone from fire has been described, but the conditions and causes that produce cracking, checking, transverse splitting, warping, longitudinal fractures, curved transverse fractures, straight transverse

fractures, patina fractures, and delamination fractures of burnt bones are not completely understood. Many characteristics, including the freshness of the bone, the amount of soft tissue, temperature of the fire, and duration of exposure cause variation in the expression of the diagnostic characteristics of fire damage.

Twenty deer humeri were used in the experiment: 16 experimental and 4 controls (Control A: burned but no blunt force trauma; Control B: blunt force trauma but no burning). Defleshed deer humeri were chosen as a model given their local availability and similarity in cortical thickness to human humeri (white-tailed deer proximal/distal mean: 3.1 mm, 3.5 mm, human proximal/distal: 3.6 mm, 3.9 mm). Radiographs and photographs were taken before and after trauma infliction, at 10 minute intervals during the burning process, immediately after burning, and following reconstruction. The experimental humeri were suspended at both ends, fractured with a lead pipe, and burned in a fire pit for a minimum of 120 minutes at a temperature of at least 200°C. To create a more realistic scenario, common fuels and accelerants were used including hickory wood chips, newspaper, and an ethanol-based lighter fluid. Temperature recordings were taken every 10 minutes with an EDL® E-Z Probe pyrometer with an air cage probe to ensure the fire was above 200°C. The temperature of the fire fluctuated throughout the burning period. The observed temperature fluctuations were reflected in the variation in discoloration on the bones. All ash was screened after burning for maximum recovery. Each humeri was independently described by the second and third author and compared. The temperature and duration of the fires were modeled against the final morphological state of the humeri using ordinal and logistic regression.

Comparison of both the radiographs and descriptions of the five major heat-induced fracture patterns (longitudinal, patina, delamination, curved transverse, and straight transverse) to the traumatic fracture patterns observed in the experimental humeri illustrates the fundamental morphological differences between traumatic and heat-induced fractures that are distinguishable in a forensic context. When a blunt impact causes a butterfly fracture, the sharp and symmetrical fractures remain distinguishable following burning. In contrast, heat-induced fractures rarely traverse the bone and instead create short segments that flake off during the burning process. Recovery yield after burning affected the number of features available to describe; moreover, humeri with lower recovery yields had much more ambiguous descriptions and diagnoses.

**Blunt Force Trauma, Burned Bone, Radiograph**

## H38 A Forensic Pathology Tool to Predict Pediatric Skull Fracture Patterns - Part 1: Investigations on Infant Cranial Bone Fracture Initiation and Interface Dependent Fracture Patterns

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The goal of this presentation is to inform attendees about the initial findings of fracture pattern analysis studies caused by impulsive loading of the parietal bone in a developing porcine (pig, *Sus scrofa*) model of the human.

This presentation will impact the forensic community by describing the expected fracture patterns and sites of fracture initiation in the developing porcine model. It will also serve as a prototype for future skull fracture modeling efforts for the pediatric human victim.

In fatal cases of violence against infants and young children, it is the job of the forensic specialist to determine the true cause and manner of death. While this is sometimes possible by establishing a "history of abuse," in other instances the ability to determine child abuse from accidental injury may be difficult. Because there is a lack of skull fracture standards for infants and young children, pediatric deaths involving single event head injuries with associated cranial fractures represent one of the greatest challenges to forensic pathologists and anthropologists.

The ultimate goal of this multiphase, interdisciplinary research project is to develop and validate a computational human head model that will predict skull fracture patterns in the human pediatric skull for a variety of impact interface conditions. Such data may be gathered from witnesses, defendants, and investigators in any given crime scene setting. Unfortunately, the ability to produce experimental skull fracture data is limited by ethical considerations surrounding experimentation on human pediatric cadavers, even if available tissue were to exist. Thus, the porcine head is being used to develop a computer-based technology that may ultimately help predict various skull fracture patterns in human infants and young children as a function of age, impact velocity, energy, and interface condition.

Using an experimental facility in which an impact mass is dropped from a specified height onto the parietal bone with different interface conditions, fracture patterns and sites of initiation have been collected on more than 80 porcine specimens. In order to understand the mechanisms of skull fracture to be incorporated in the computational model, it is important to first document sites of fracture initiation. All specimens were impacted in the central parietal region at an energy level that caused the initiation of a linear fracture. The locations of initial fracture (in the order of decreasing frequency) were: on the parietal bone at, and perpendicular to, the lambdoidal suture; on the parietal bone at, and perpendicular to, the coronal suture; on the frontal bone at, and perpendicular to, the coronal suture; and on the frontal bone at the superior orbit and parallel to the coronal suture. The results of this study showed that in the porcine model all major fractures on the cranium initiate away from the point of impact at the parietal suture margins, and radiate back toward the center of the parietal. This phenomenon has occurred with regularity regardless of the type of interface (rigid or compliant).

The fracture patterns caused by the different interfaces (rigid and compliant) were different and varied with animal age. On the skulls of animals aged under seven days there is significantly more suture damage caused by the compliant interface than by the rigid interface, which may be a function of the suture and bone properties in these young specimens. Another significant finding at this point in the study seems to be that there is more overall skull damage being generated in impacts from the same height with a compliant surface (carpeting, sod, etc.) versus a rigid interface (concrete, wood floor, etc.).

These results identified multiple fracture initiation sites on the porcine skull away from the impact site and showed that the compliant interfaces caused relatively more fracture damage to the developing porcine skull than did rigid interfaces. The next phase of the project will be to elaborate on these results, specifically investigating energy dependent fracture propagation, and developing a scaling scheme to compare anatomical growth patterns in the human skull and the porcine model.

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**Child Abuse, Bone Fracture Patterns, Bone Biomechanics**

# H39   And a Little Child Shall Lead Them....

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After attending this presentation, attendees will recognize the limitations of presently available skeletal aging standards for infants and young children.  Attendees will also be made aware of some of the approaches utilized in assessing abuse in immature skeletal remains.

This presentation will impact the forensic community by emphasizing the need for caution when applying currently available immature skeletal age standards to contemporary forensic cases, and hopefully, encourage the acquisition of more appropriate skeletal data from present day populations of varying ancestry and socioeconomic backgrounds.

In past cases involving child abuse, the authors' primary focus has been on analyzing trauma.  The chronological age of the victim at death has always been known.   In 2007, a request was made for a determination of age at death, as well as a review of the remains for possible trauma.  The parents of the dead infant gave conflicting stories in regard to when the child had died.  Relatives were similarly uncertain.

Examination of the dentition provided a dental developmental age estimate of 12-20 months.  While attempting to establish a skeletal developmental age, the paucity of data for this portion of the life cycle was noted (see Scheuer and Black 2005 for a summary of the little that is available).  Based on the available data, the skeletal developmental age estimate for this child appeared to be 12-24 months.  Although the estimated skeletal developmental age was consistent with the dental developmental age, it was apparent that the data used for the age estimate were from children of an earlier time, as well as different ancestry and socioeconomic status.

These concerns regarding the lack of appropriate data were further emphasized upon attempting to use long bone diaphysial length age data to confirm the dental and skeletal developmental age estimates.  Most available dry bone data were based on archaeological individuals whose so-called "known age" was based on dental developmental age estimate.  A few were from historic cemetery collections with ages from cemetery records.   Radiographic bone length data from immature living individuals of known age at death were scarce and from inappropriate populations ("white", "middle class").  The child in this case was "black" and "lower class."

For example, the radial and tibial diaphyseal lengths, when compared with the tables in Gindhart (1973 AJPA 39: 41-48) are consistent with those of a much younger child (radius: 8-12 months, tibia: 6 months).  The Gindhart series consists of males and females of a mainly white middle class background from the Fels Research Institute Longitudinal Growth Study in Yellow Springs, Ohio that began in 1929.  The time period for Gindhart's sample is 1930-1967.  Comparison with tables in Ruff (2007 AJPA 133:698-716) yielded ages at the lower end of the range for 12 months (humerus, radius, tibia) and under the range for 12 months (femur).  Ruff utilized Denver Growth Study data collected from a primarily white middle/upper middle class.  It should be noted that more recent and appropriate samples are not available.

The observed secular increase in stature during recent years magnifies the significance of the comparative "shortness."  Given the above limitations, the long bone lengths of this individual, lagging well behind skeletal and dental developmental age, are suggestive of growth deficiency due to malnutrition and/or disease.

The possibility of malnutrition and disease was further reinforced by the radiographic presence of multiple lines of increased density ("growth arrest," or "Harris" lines) at the growing ends of several long bone shafts (distal tibia, femur, fibula, radius).   Growth arrest lines represent a cumulative deposition of bone salts laid down during periods of protein deficiency as growth of metaphyseal cartilage is reduced.  Causative factors may include malnutrition and/or disease and/or abuse.

Traumatic child abuse was specifically suggested by the discovery of healed "bucket handle" (or "corner") fractures of the distal tibiae, visible only in radiographs.  These fractures, which differ from "normal" toddler injuries, are strongly associated with child abuse.

*Technical Note:*  Because full body standard x-ray equipment was not immediately available (the office uses fluoroscopy), a portable, digital dental x-ray unit routinely used by forensic odontologists provided excellent radiographs.  This machine has also been used in child abuse cases involving rib fractures.   The resulting digital radiographs of these small and often difficult to image bones are easily acquired and of very high quality.

There is a great need to gather more appropriate contemporary immature skeletal data.  Due to continuing concern regarding x-ray dosage in the living, it would be helpful if forensic practitioners would obtain and share x-ray studies of immature individuals whose chronological age at death is known.  Warren (1999), for instance, has done so for fetuses and stillborns.  This approach should be extended into early childhood.

**Child Abuse, Infant Skeletal Age, Radiology**

# H40   Callus Treatment:  Collaboration Between Forensic Anthropology and Forensic Pathology to Improve the Recognition and Elucidation of Skeletal Fractures in Infants and Children

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After attending this presentation, attendees will gain an appreciation of the significance of cooperation between disciplines in the medicolegal arena and how this cooperation can improve the quality and scope of investigations into child death.  When forensic anthropologists and pathologists combine their skills and expertise, the result is a more thorough and complete investigation.

This presentation will impact the forensic community by demonstrating that increased cooperation between disciplines results in a better understanding of complex forensic cases, in particular those involving suspected child abuse cases.

The syndrome of child abuse (first defined and its causes identified by Kempe in 1962) refers to cases in which children are beaten, burned, shaken, thrown, hit, or dropped.  These actions may result in the child's death.  Without physical abuse, fractures in infants and young children are rare.  When children become active in school and sports, around the age of five, they develop greater risks for accidental skeletal fractures.  Most skeletal fractures related to child abuse occur in children younger than 18 months.  Repeated fractures in varying stages of healing may be present in the same infant; when this occurs, the term "battered child syndrome" is applied.  In these cases, a complete understanding of the fracture pattern, timing, and stage of healing for each fracture is critical.  Collaboration between the pathologist and the anthropologist can enhance this understanding as demonstrated by the cases discussed below.

On January 30, 2008, a three-month-old male child was admitted to the hospital after sustaining injuries due to suspected child abuse.  He was transported from a county in Northern Arizona to a regional trauma center in Phoenix, Arizona where radiographs and blood tests were obtained.  The infant subsequently died from his injuries.  An autopsy was performed at the Maricopa County Office of the Medical Examiner.

The antemortem radiographs were obtained and they revealed the presence of multiple fractures, including comminuted, healing fractures of the left humerus and multiple ribs. The humerus and exemplars of the ribs were removed at autopsy for maceration and examination by the anthropologist. After a subsequent consultation and further examination, the pathologist and anthropologist removed the right innominate and the remainder of the rib-cage, all of which were macerated.

After maceration, additional radiographs were obtained to assist further examination. These radiographs, as well as those obtained prior to the autopsy, were used to place the ribs into anatomical order so that the injuries could be enumerated by joint effort of the pathologist and the anthropologist. The findings of osseous traumata were as follows: the right ribs had 14 well-established calluses, 3 acute, re-fractured calluses, 1 traumatized callus, 1 acute, non-healing fracture, and elaboration of bone on the sternal end of the first rib. On the left ribs there were 13 well-established calluses, 5 acute re-fractured calluses, and elaboration of bone on the sternal end of the first rib. The left humerus had a well-established callus, which encompassed nearly three-quarters of the distal shaft, with two complete fractures of the shaft; one fracture was grossly visible and the other was embedded in the callus. The superior ramus of the right *os coxa* exhibited one less well-established callus and one acute injury immediately lateral to the callus with associated hemorrhage. Additional fractures were noted on the radiographs.

Several other complex cases involving acute, healing, and healed fractures of the ribs and long bones in infants under the age of one year also resulted in maceration and examination of the injured bone by both the anthropologist and pathologist. In all cases, maceration allowed for better follow-up radiography and examination with a dissecting microscope to enhance understanding of the injuries. These cases will be used to demonstrate that collaboration between the forensic pathologist and forensic anthropologist can lead to a higher degree of understanding for complex cases involving suspected abuse of children.

**Interdisciplinary Collaboration, Skeletal Traumata, Suspected Child Abuse**

## H41 Eaten or Attacked By His Own Dogs? From the Crime Scene to a Multidisciplinary Approach

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The goal of this presentation is to encourage attendees to use all the resources they may have to solve difficult cases of decomposed bodies or human remains they may encounter. These resources include: different experts, techniques, and ancillary examinations.

This presentation will impact the forensic community by appealing for multidisciplinary approaches at the crime scene including various areas of expertise (e.g., entomological, pathological, or other areas of expertise such as veterinary in this case), as the key to answer the medico-legal questions often presented by decomposed bodies. Additionally, emphasis is given to the importance of the presence of a forensic anthropologist or forensic pathologist at the crime scene investigation.

Although the presence of a forensic pathologist (FP) at a crime scene it is not uncommon, a forensic anthropologist (FA) coming to the scene in routine cases can be considered a rarity in most jurisdictions. However, their usefulness is undeniable and sometimes represents a unique source of information to understand the circumstances of death. A multidisciplinary approach is another requisite frequently recommended for the autopsy of bodies in different states of decomposition. Occasionally, more experts than the usual FA and FP are necessary, as in the case presented here.

Part of a decomposed human body from an individual, missing for one and a half months, was found locked in his home along with the decomposed bodies of the three dogs that lived with him. The rooms were in disarray with overturned furniture, signs of intense movement and dog excrement everywhere. No weapons or other evidence of a struggle were found. All the bodies were recovered for autopsy.

The human cadaver was reduced to the right humerus, scapulae, pelvis, and distal lumbar vertebrae. The remains were mainly skeletonized with the lower limbs showing mummification with some adipocere. The disarticulated skull (actually only the cranial vault) was lying on the floor two meters away from the remainder of the body. Multiple larvae continued their work on the human remains and pupae were also present. The long bones and particularly the scapula showed typical animal bite marks consistent with dogs. The skull, apart from the tooth marks, also exhibited a regular round defect on the anterior and middle cerebral fossas which may have resulted from dog clawing. All the facial bones and mandible were absent. Dog hair was spread all over the trousers of the victim.

Positive identification was achieved through anthropological features (Caucasoid, short stature, male, 55-60 yrs.) and subsequent confirmation by DNA analysis. Furthermore, entomological analysis estimates time of death to be around 1 month before discovery.

Because no peri-mortem injuries were found, cause of death could not be assessed. Furthermore, the disappearance of half of the body was difficult to explain. It was decided to autopsy the dogs and the collaboration of a veterinarian was requested. The dog cadavers were putrefied, and a cause of death could not be ascertained: no traumatic injuries, cancer, or other noticeable conditions were found. But in one of the dog's stomachs were small fragments of bone, most likely from the hand bones and vertebrae of the man.

These features, and their relationship with the crime scene investigation and police information, are discussed. Taking all the elements into account, it is concluded that the man likely died of natural causes and later he was eaten by his own famished dogs. The pattern of the bone lesions are discussed and are compared to the dog's characteristics.

This case shows the importance of the crime scene investigation. If this examination had not been undertaken, the understanding of cause and manner of death would have been extremely difficult, pointing out the necessity of both FA and FP being present at the scene. Finally, a multidisciplinary approach, in this case integrating the FA, the FP, police, veterinarians and entomologists, is the key to answer the questions that decomposed bodies often generate.

**Eaten, Dogs, Death**

## H42 Solving Medical Examiner Cold Cases: Modern Resources in the Reanalysis of Human Skeletal Remains

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After attending this presentation, attendees will have a better understanding of the processes involved in the reanalysis of unidentified remains that may lead to positive identifications, removal of non-forensic cases from national databases, and the reanalysis of skeletal trauma often essential in the adjudication of homicide trials.

This presentation will impact the forensic community by providing standard operating procedures that prioritize the reanalysis of trace evidence from medical examiner cold cases through the effective use of state-of-the-art technologies in the following fields: anthropology, DNA, fingerprinting, odontology, facial reconstruction, tool mark analysis, digital photography and documentation, and the productive use of missing persons databases (e.g., NCIC, NamUS, NCMEC, NCMA, CODIS, FLUID DB, and other databases).

Medical examiner cold cases often remain in dry storage or refrigeration for years to decades without the benefit of reanalysis. However, President Bush's DNA Initiative, the Joint Initiative of the Florida Department of Law Enforcement Missing Children Information Clearinghouse, and the Florida Medical Examiner's Commission have helped to re-focus an over-taxed medical examiner system and underscored the importance of medical examiner cold case reanalysis. To this end, this study presents cold casework from Florida Medical Examiner Districts 4 and 20. The pairing of these two medical examiner districts was prudent because of the disparity in case loads between these two entities (e.g., Homicides District 4 = 148; Homicides District 20 = 5) and because they share a forensic anthropology consultant. Additionally, District 20 provided supplementary evidence storage space for District 4 and quick access to Florida's Unidentified Decedent's Database (FLUIDDB) which was housed therein.

For this study, the standard operating procedures for the reanalysis of unidentified remains and those remains retained for trauma analysis was in accordance with Florida State Statute 406.11. As such, the following standard order of operations was applied (when applicable): collection/resubmission of blood and fingerprint cards, odontology charting and radiography, exhumation, metric and non-metric anthropological analysis, and 2-D or 3-D facial reconstructions. These new data were input into the following databases: NCIC, NamUS, NCMEC, NCMA, CODIS, AFIS, and FLUIDDB databases.

Data were pooled from the eighty-seven medical examiner cold cases in order to identify trends in the sample (e.g., peaks in numbers of unidentified, completeness of remains, and biological profiles). Results show that the lowest numbers of unidentified remains occurred from 1974 to 1979 which represented 7% (n=6) of the sample. The number of unidentified remains peaked between 1985 and 1989 (31%; n=27). Lastly, the numbers of unidentified remains was constant from 2000 to 2004 (15%; n=13). These findings were compared to statewide and national trends presented in the Bureau of Justice Studies' 2007 Fact Sheet (a public domain document). Florida's numbers of unidentified peaked from 1980-1984 (24%; n=230) and had the fewest numbers of unidentified from 1990 and 1994 (17%; n=157). Florida's numbers of unidentified has continued to rise for the last 15 years. The national trend in numbers of unidentified remains was lowest from 1980 to 1984 (15%; n=1,516), peaked from 1990 and 1994 (26% (n=2,686) and remained constant from 1995 to 2006 (19%; n=1,956). Interestingly, the research sample was in keeping with the nationwide trend in numbers of unidentified remains when analyzed by 5 year periods. However, while Florida's statewide numbers of unidentified continue to rise, the sample compiled for this study continues to drop as a result, in part, of our reanalysis of medical examiner cold cases.

The numbers of non-forensic and forensic cases within the sample were also noted. The authors observed 15 (17%) archaeological cases (e.g., the decedent was dead for greater than 75 years; FL statute 872.05). There were 2 anatomical specimens (2%) as evidenced by screws and plasticized veins and arteries, and sixty-seven cases (77%) were of forensic significance. Of the medical examiner cold cases presumed to be forensic and unidentified, at least five (6%) were isolated mandibles and maxillae, 22% (n=19) represented nearly complete skeletons, 37% (n=32) were comprised largely of long bones and postcrania, and 57% (n=50) had a cranium or cranial fragments present.

Through the analysis of these cases, this research emphasizes the various challenges facing the procedures used to solve medical examiner cold cases. Thus far, significant strides have been made in the resolution of medical examiner cold cases through the (1) modernization of standards in several forensic disciplines, (2) the effective use of missing persons databases, and (3) the collaboration of multiple agencies and resources.

**Cold Cases, Missing Persons, Forensic Anthropology**

## H43 What Lies Beneath: Re-Examining a Cold Case Homicide From a Forensic Anthropological Perspective - A Case Report

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The goal of this presentation is to bring continual awareness to the need for collaboration in the various fields of forensic science such as forensic pathologists and forensic anthropologists.

This presentation will impact the forensic community by showing how a recent collaboration between the medical examiner and forensic anthropologist revealed significant new evidence that warranted the revision of the crime reconstruction.

Within the last decade in the United States forensic anthropologists have become more prominent participants in the analysis of cases that are brought to the medical examiner's office. Forensic anthropologist's participation often leads to the discovery of significant information that aids in the identification of an individual and/or aids in the medical examiner's opinion of death. Now, with supported efforts by medical examiners, well-trained forensic anthropologists located in proximity to or within medical examiner's offices are re-examining human remains from numerous cold cases to perhaps discover additional evidence previously not found.

This case presentation gives a strong argument for the need for collaboration between various forensic experts, such as the forensic pathologist and forensic anthropologist. This case report shows how the

forensic anthropological analysis disclosed significant additional evidence in this homicide case. Initially, the external examination revealed the predominance of sharp force trauma; however, after the anthropological examination it was clear that blunt force trauma was equally prevalent. The anthropological report was significant enough to warrant changes in the reconstruction of the activities of the crime.

In 2007, the present Chief Medical Examiner of Galveston County, Texas requested an anthropological examination of the desiccated remains of an unidentified individual whose medical examiner's report fourteen years ago stated cause and manner of death as homicide by multiple stab wounds. Multiple stab wounds were found on the desiccated tissue of the neck, chest, and abdominal regions. The medical examiner's opinion of death is not refuted by the present Chief Medical Examiner; however, it was believed identification information or evidence of additional trauma could be obtained by a more detailed analysis of the skeletal remains.

A detailed examination revealed that, in addition to multiple stab wounds, the individual had multiple bone fractures as a result of blunt force injuries. Fractures were identified on the mandible, hyoid bone, mineralized portion of the thyroid cartilage, left and right ribs, cervical and thoracic vertebrae, left scapula, the ilio- and ischiopubic rami of both *os coxae*, and the sacrum. An excessive amount of force is required to produce fractures of both the sacrum and the *os coxae* and, typically, massive intra- and retroperitoneal hemorrhaging occurs. The comminuted fracturing of the pubic bone and both rami can be categorized as crushing fractures and have been noted in vehicular impacts. Cervical vertebrae fractures are also associated with violent force. This additional information was of significant importance in the continual resolution of the case and is another example of the synergistic relationship needed between forensic pathologists and forensic anthropologists in the analysis of human remains in the later stages of decomposition.

**Forensic Anthropology, Forensic Pathology, Blunt Force Injury**

# H44    Age-Related Change in Adult Orbital Shape

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After attending this presentation, attendees will better appreciate the impact of age on adult orbital shape.

This presentation will impact the forensic community by highlighting the importance of considering age when utilizing orbital shape in the identification of unknown remains.

The orbital region conveys a wealth of information about sex, ancestry, and age. This is thanks in large part to its topological complexity, which is a consequence of soft and hard tissue interactions. With age, the smooth appearance of youthful skin is replaced with deepening lines of demarcation (e.g., crows' feet) as the soft tissue around the orbits descends. As interrelated entities, internal hard tissue (i.e., bone) modification in this region may impact the manifestation of external soft tissue change over time. However, alterations in craniofacial bone structure post-adulthood are not as well understood as those witnessed on the soft tissue level. At present, adult orbital shape is known to vary both metrically and non-metrically with sexual dimorphism and ancestry (Krogman 1962; Rhine 1990; Bass 1995), but the impact of the aging process on this region remains a relative mystery (Williams 2008).

The present study addresses this complex issue by applying three-dimensional semi-landmarks to the orbital rims of 664 crania embodying a mix of socially-determined race (Black, White) and biologically-determined sex from the Terry, Hamann-Todd, Maxwell Museum, and W.M. Bass skeletal collections of known individuals. The crania were parsed into three overarching age groups (young adult: 18-39 years; middle-aged adult: 40-59 years; elderly adult: 60+ years). In craniometric analyses, semi-landmarks are often employed in regions lacking distinct landmarks, such as boundaries and surface curvature. Superior and inferior orbital rim curvature was gathered as continuous stream data using a portable digitizer. Semi-landmarks were then extracted utilizing a beta program (Slice 2005), which applies an algorithm that re-samples each curve into a user-defined number of evenly-distributed points (10 points per curve; four curves).

The resultant semi-landmark data were fit into a common coordinate system via a generalized Procrustes analysis (GPA), which filters out the effects of location, scale, and rotation. In order to reduce dimensionality, a principal component analysis (PCA) was performed on the covariance matrix of the GPA-aligned coordinates and the resulting principal component (PC) scores, which accounted for 95% of the total variance, were utilized in subsequent multivariate statistical analyses. A multiple analysis of variance (MANOVA) of the PC scores detected a significant age effect (F=1.98; df= 64; Pr>F=<0.0001), as well as a sex*age (F=1.43; df=64; Pr>F=0.0156) and race*sex*age interaction (F=1.65; df=64; Pr>F =0.0012). The specific age groups contributing to the three-way interaction were evaluated by conducting contrast tests, which compared the age group means for each subpopulation (e.g., White females) within MANOVA. Contrast tests found several significantly different age pairings in the subpopulations (Black males: young versus elderly F=2.59; df=30; Pr>F=<0.0001, White males: young versus middle-aged F=1.94; df= 29; Pr>F=0.0058; young versus elderly F=1.90; df=29; Pr>F=0.0078, White females: young versus middle-aged F=1.63 ; df=27, Pr>F=0.0415, middle-aged versus elderly F=2.10; df=27; Pr>F=0.0038). These age-related orbital differences were visualized in terms of spatial distinctions using vector plots which compared the mean shapes between age pairings that were found to be significantly different. While a specific pattern did not always manifest in these plots, the eye orbits did exhibit such shape changes as supero-inferior expansion, supero-inferior compression, and medio-lateral compression depending on the subpopulation and age groups analyzed.

These results indicate that adult skeletal orbital shape does in fact change with the aging process. Moreover, orbital shape is influenced by the interaction between age, sex, and ancestry. Thus, it is inappropriate within a forensic context to treat the orbital shape differences typically associated with sexual dimorphism and ancestry as static. Instead, age should be factored into sex and ancestry determinations of unknown remains which rely on orbital shape.

**Eye Orbits, Semi-Landmarks, Geometric Morphometrics**

# H45    Craniofacial Growth, Maturation, and Change:  Teens to Mid-Adulthood

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After attending this presentation, attendees will learn that the craniofacial skeleton attains adult size and shape at a much younger age than previously assumed.

This presentation will impact the forensic community by adjusting current perceptions in regards to the age at which adult craniofacial dimensions are reached.

The different structural units which constitute the craniofacial complex develop and grow under differential mechanical forces. Replacement of cartilage to bone, sutural deposition, and periosteal

remodeling are the three principal processes which drive craniofacial skeletal growth. The bones of the cranial base develop through endochondral ossification, which is preceded by a hyaline cartilage precursor largely confined to the spheno-occipital synchondrosis. The vault, facial, and mandibular bones, per contra, develop through intramembranous ossification or tissues that are of neural crest origin.

The neurocranium develops earlier and faster than the craniofacial skeleton in newborns and infants, approaching adult size and configuration by age 10. The slower growing craniofacial skeleton is influenced by tooth eruption, which drives alveolar process development. Thus, an increase or decrease in tooth number or tooth loss during growth will have a direct effect on the shape of the facial skeleton. Craniofacial growth is thought to cease around 17 years of age following eruption of the permanent dentition

Strand Viðarsdóttir and colleagues (2002) found that population-specific facial morphologies are present at birth regardless of sex and are modified during ontogeny, thus suggesting that ancestry can be determined in subadults. Meanwhile, in their cephalometric study of craniofacial changes in the third decade of life, Akgül and Toygar (2002) found significant changes, which were more pronounced in women and the lower region of the face. In order to further examine developmental shape and size differences in the craniofacial skeleton and the potential for the identification of subadults, as well as their possible inclusion in population studies, the present study used geometric morphometric methods to compare individuals from a single population partitioned into four age groups: 14, 16, 20, and 25+ years. Twenty-six type 1 and type 2 standard coordinate landmarks were used in this study. The sample consists of 4 fourteen-year-olds, 5 sixteen-year-olds, 3 twenty-year-olds, and 12 twenty–five-year-olds and is derived from an African slave population housed in the Morton Collection at the University of Pennsylvania. After GPA superimposition of the raw coordinates, the resulting shape variables and Centroid Size were utilized in the subsequent multivariate analyses. A multivariate analysis of variance (MANOVA) test using the first ten principal component scores corresponding to 83% of the total variance detected significant shape differences among the age groups ($F$ = 1.92; df = 30, 32.96; $Pr > 0.0355$). In addition, the "contrast" statement d in the proc GLM procedure was used to detect the specific groups that differed. Surprisingly, the groups that differed significantly were the 20 and 25+ age groups ($F$ = 3.64; df = 10, 11; $Pr > 0.0224$). In addition, a Pearson Correlation Coefficient was used to examine the relationship between shape variation using the Principal Component scores and Centroid Size. No significant correlation was detected between the PCs and Centroid Size for the sample as a whole, meaning no significant scale differences were detected. These results suggest that there is no significant shape or size differences between older subadults in their mid teens and adults, thus signifying that subadults reach their final form earlier than expected. The significant difference between 20-year-olds and the over 25 group concurs with the study by Sarnas and Solow (1980) who found displacements in nasion and sella between 21 and 26 year olds. The changes between the 20 and 25+ age groups are probably multifactorial in nature and related to the eruption of the third molars and/or possibly alveolar remodeling due to the aging process. These studies have clinical as well as forensic implications for the identification of subadult and adult crania.

**Craniofacial Growth, Teens, Adults**

## H46 Estimating Advanced Adult Age-at-Death in the Pelvis: A Comparison of Techniques on Known-Age Samples From Iberia

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The goal of this presentation is to demonstrate the difficulty of estimating age in elderly individuals by comparing the performances of four age-estimation methods on skeletal samples of advanced age. This study examines factors undermining successful age estimation, and investigates whether new techniques may have the potential to advance the study of aging in elderly individuals.

This presentation will impact the forensic community by exploring the utility of various aging techniques to the forensic anthropological community - specifically, by testing and comparing traditional aging techniques with a new Bayesian method of age estimation that may prove valuable to the forensic anthropologist. The presentation focuses on the as-yet-unsolved problem of estimating advanced adult age. Thus, it will provoke discussion on whether accurate age estimates for the elderly are a worthwhile goal or a biological impossibility, and stimulate future research on the forensic applicability of both new and traditional methods of age-at-death estimation.

An accurate adult age-at-death estimate is the goal of much forensic anthropological research; it is a crucial component of the biological profile that facilitates skeletal individuation and victim identification. However, accurate age estimates into the decade of the 60's and beyond are rarely achieved with current methods. Historically, morphological age-at-death estimation techniques have focused on the articular surfaces of the bony pelvis. The Suchey-Brooks pubic symphysis method, and the Lovejoy et al. and Buckberry and Chamberlain auricular surface methods, have been tested on many populations worldwide. However, even these widely-used techniques can result in estimation errors and broad age ranges that prove forensically uninformative. Because of the irregularity of skeletal degenerative change, such errors are often exaggerated in individuals of advanced age.

These three methods are tested on modern Iberian skeletal samples of known age from the Universitat Autònoma de Barcelona (N=34) and the Universidad de Valladolid (N=80) which include an abundance of elderly individuals (>60 years) and therefore highlight the hazards of applying traditional age estimation techniques to individuals of advanced age. The Rissech et al. method, a Bayesian age estimation technique based on the acetabulum, is also tested. When tested on these samples, the three traditional methods perform with similar but high degrees of inaccuracy and bias, sometimes overestimating the ages of young adults by up to 20 years and underestimating the ages of older adults by up to 35 years. In estimating the ages of elderly Iberian individuals, the three methods perform equally poorly. In contrast, the Rissech et al. method estimates male age with significantly lower inaccuracy and bias than do the three traditional techniques. In addition, its population-specific point age estimates and narrow confidence intervals suggest its utility to the forensic anthropologist.

Differences in ancestry, activity levels, and rates of skeletal degeneration continue to make skeletal estimation of advanced adult age problematic. However, accurate age estimates and biological profiles, even for the elderly, remain a goal for forensic anthropologists. These results suggest that Bayesian techniques like the Rissech et al. method may have the potential to improve age estimation among elderly individuals.

**Age-at-Death, Pelvis, Iberia**

## H47 The Sacral Auricular Surface: A New Approach to Aging the Human Skeleton

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After attending this presentation, attendees will appreciate the potential of the sacral auricular surface as an additional means of age estimation. A different, customized approach to aging the human skeleton will be presented along with its potential.

This presentation will impact the forensic community by presenting a new means of estimating age from the human skeleton and a new methodology that has the potential, with further research, to lead to a more individualized approach for estimating age at death.

This study proposes to identify and define morphological characteristics of the sacral auricular surface that correlate with age. Character selection began with a survey of those described by Lovejoy et al. (1985) and culminated with the refinement of modified characters. These were then tested for correlation with age using the Robert J. Terry Anatomical Skeletal Collection (n=410), housed at the National Museum of Natural History. Corresponding sacral and iliac surfaces were evaluated for reciprocal age changes; however, none were identified.

The characteristics defined for the sacral auricular surface are modifications of those described by Lovejoy et al., but also include new characters specific to the sacral auricular surface. Billowing, striations and transverse organization as described by Lovejoy et al. were omitted as they did not appear on the sacrum. Surface characters adapted from Lovejoy et al. include granularity (three grain types are observed on the sacral auricular surface) and retroauricular activity (surface characteristics differ significantly from Lovejoy et al.). Microporosity, macroporosity, and density were adapted without change from Lovejoy et al.; however, a new scoring system was devised for the expression of these characters. Newly devised characteristics involving the border of the auricular surface include thickened, arthritic sharp and arthritic lipped margins.

The sacral auricular surface was divided into four regions or quadrants. Each surface characteristic was then defined individually for each region and assigned a score based on presence, absence and degree of expression in keeping with Buckberry and Chamberlain (2002). The division of the sacral auricular surface into quadrants allowed an evaluation of the progression of degenerative changes over the entire sacral auricular surface.

The aging method developed in this study involves bracketing whereby the minimum and maximum ages of occurrence of particular characters define the upper and lower limits of the estimated age interval. This approach is more individualized, allowing the examiner to delimit an age interval based upon surface characters specific to the sacrum being examined, as opposed to identifying a phase with a set age interval. For example, traits that do not occur before a certain age can be used to set the lower limit to the age interval (e.g., a score of 1 for microporosity in region 1 does not occur before age 22) and traits that do not occur after a certain age can be used to set the upper limit (e.g., a score of 3 for primary grain in region 2 does not occur after age 38) resulting in an individualized age interval, of 22-38 years. This is particularly useful in instances where a surface may be in between two classically defined phases.

This aging method was tested using the William M. Bass Donated Collection (n=100), housed at the University of Tennessee, Knoxville. Results indicate accurate placement of individuals in 70% of the cases. Although not particularly high, accuracy may be improved with further research and incorporating other established aging methods to this individualized method of age estimation. The age ranges formulated from this customized method are from five to fifty years and are particularly useful in aging older individuals, even beyond the fifth decade. Additionally, the sacral auricular surface method, when used in conjunction with other established aging methods, may be of value in narrowing large age ranges.

**Sacral Auricular Surface Aging Method, Age at Death Estimation, Forensic Anthropology**

## H48 Cranial Suture Closure as a Reflection of Somatic Dysfunction: Lessons From Osteopathic Medicine Applied to Physical Anthropology

*Anne M. Kroman, PhD\*, and Gregory A. Thomspon, DO, Lincoln Memorial University, De-Busk College of Osteopathic Medicine, 6965 Cumberland Gap Parkway, Harrogate, TN 37752*

After attending this presentation, attendees will have a great appreciation of anatomy and physiology of the cranium and potential factors that may influence adult cranial suture fusion.

This presentation will impact the forensic community by drawing attention to potential hazards of utilizing a well-known method of determining age in adult skeletal remains.

The construction of an accurate biological profile has long been a cornerstone of physical and forensic anthropology. Among the diverse components of the analysis, the correct determination of age at death has long been considered one of the most challenging. Adult, or degenerative aging, can be fairly ambiguous with large ranges in variation, as seen by the large ranges of standard deviations in a majority of the methods. The foundation behind estimation of age at death determined from mature skeletal remains is anchored on the core principle that the degenerative changes are both uniform and predictable.

One of the most controversial methods of adult age estimation is assessment of cranial suture fusion. Since its conception and application, the technique has been fraught with controversy. Initially, two "rival" schools of thought existed regarding sutural ossification; that they were either a normal progression of age or the manifestation of pathological condition (Hershkovitz et al 1997). The former theory prevailed in the literature, and the work of Todd and Lyon set the standard for utilizing cranial suture ossification as a viable method for aging adult skeleton remains in physical anthropology (1924). However, several issues regarding the methodology and analysis of some of the earlier techniques have recently been called into question (Hershkovitz et al. 1997, Meindl and Lovejoy 1985). Numerous researchers have attempted to refine the method and identify possible confounding factors, with varied results (e.g., Hershkovitz et al. 1997, Nawrocki 1998, Zambrano 2005).

The "confounding factor" at work may be no other than the basic principles of anatomy and physiology of the cranium. The regard for the skull as a static, immobile entity is a view point that is long overdue an intellectual overhaul. Joints, by inherent nature, are designed to provide movement to some degree, and the cranial sutures are no exception. They, like all joints, will remain patent as long as there is motion, and fuse only when the motion has ceased. While the degree of motion present between the cranial bones is small, it has been well documented in medical and neurological studies (Heisey and Adams 1993). It is also noted that severe changes to the skeleton, or somatic dysfunction, can have an effect on the patency of the cranial sutures and may lead to pathological fusion.

Cranial suture fusion and obliteration is best explained as a mechanism of somatic dysfunction rather than a linear, predictable

degenerative consequence of aging. To assess the effect of skeletal somatic dysfunction on the fusion of cranial sutures, analysis was conducted utilizing the William M. Bass donated skeletal collection housed at The University of Tennessee. From the collection 100 individuals of known age, race, and sex were randomly selected for inclusion. The analysis was conducted in two phases. In the first phase, each of the crania was scored for cranial suture closure following the methodology outlined in Meindel and Lovejoy (1985). The second phase included a more in-depth analysis of the cranial and post cranial skeletal remains by a physician. The strain pattern, if present, was identified and scored for each crania, along with other areas of somatic dysfunction in the skeleton (e.g., sacroiliac fusion, ankylosing spondylitis, severe scoliosis). A stronger correlation was found between skeletal dysfunction, either cranial or post cranial, and cranial suture fusion than between age at death and fusion rates. Furthermore, the patterns of cranial suture fusion were highly correlated with the identified strain pattern in the cranium, as previously suggested in the neurological literature.

The results from this analysis clearly show a correlation between skeletal dysfunction and cranial suture obliteration, supporting the theory that sutural obliteration is under the influence of a variety of factors, and is not a simple, linear predicator of age. Physical and forensic anthropologists should be cognizant of the possibility that pathological processes may be present that could potentially alter results from age estimation by means of cranial suture fusion.

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**Age Estimation, Cranial Sutures, Suture Fusion**

## H49  A Multidisciplinary Test of the Lamendin Age Estimation Method

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After attending this presentation, attendees will be informed of the results of the first multidisciplinary test of the Lamendin age estimation method which has been described as "simple," "fast," and "easy to use" by its developer.

This presentation will impact the forensic community by providing insight into the utility of this purportedly simple method at scenes that require forensic identification. Such scenes could be in a classic morgue situation or in a large temporary facility functioning as a mass disaster response.

This presentation will summarize a study funded by the National Institute of Justice in 2006-2007 designed to test the Lamendin (1992) age estimation technique. This method puts to use single-rooted teeth to determine age at death. Lamendin and his co-authors described their technique as "simple," "fast, easy to use, and reasonably accurate" (1992: 1373). It appears to be an attractive method for predicting age at death, especially in rapid-response morgue situations, since it requires very little technology and calls for little to no damage to the unidentified human remains.

Lamendin *et al*.'s original research, as well as others' later follow-up studies, explored interobserver error and came to different conclusions. Lamendin *et al.* (1992) concluded that interobserver error was not significant, yet Prince and Ubelaker (2002) discovered that the experience of the observer can indeed affect the age assessments. Using 40 and 30 teeth respectively, Prince and Ubelaker (2002) discovered that the intra-observer test showed a mean error of 6.5 years. The interobserver test included three participants, one with some experience with the technique (profession not specified) and two without any prior exposure to the method (graduate students). There was a difference between the experienced and non-experienced results, with a maximum mean error of 13 years, and a maximum range of 0 to 37 years reported. These results indicated that the "features described on some teeth are subject to varied interpretation and this can lead to variable age estimates" (2002:116). The Prince and Ubelaker study demonstrated that there may be some difficulty practicing the method for the first time, thus experience may have an impact on results and overall accuracy.

More recently, the Lamendin method has been used in combination with other age estimation methods (Martille 2005), and has shown effectiveness for the age group between 40 and 60 years (ibid). This is notable since other methods, such as those involving the pubic symphysis or the sternal end of the fourth rib, are more applicable to younger individuals. In addition, practitioners have suggested refinements to the method to obtain more accurate results, either by adding a new dimension to measure (Prince and Ubelaker 2002) or by devising new formulae for specific single-rooted teeth (Prince and Ubelaker 2002; Sarajlic 2005).

This project differs from any done to date since it included more observers (five) and a larger tooth sample (over 150 teeth rather than a small subsample). In addition, the observers in this study differed from prior studies since they were four practicing professionals from different fields in the medico-legal community – anthropologist, pathologist, odontologist, and death investigator – as well as one forensic science master's level graduate student. Furthermore, qualitative data were gathered along with the required quantitative data. This qualitative data assisted in the understanding of discrepancies between observers and the reason for them. No prior studies make mention of collection of such data from participants.

The preliminary results suggest that the Lamendin technique has its strengths and weaknesses with users from various disciplines. Qualitative data revealed that periodontosis proved to be the most ephemeral variable to be measured. It was described as "difficult to see" or "visualize" in numerous specimens measured in this study. Quantitative data suggest that differences exist between observers (mean error of 7.9 years), and that observers had consistent difficult correctly estimating the age of younger adults, such as those in their late teens and twenties. However, this method appears to be useful and helpful between differently-trained practitioners. This would certainly be the case in a mass disaster situation when general triaging by age categories was an initial goal in the identification process.

Given these results, the application of the Lamendin method to mass disasters with high numbers of unidentified victims may be recommended. The lack of damage done to remains, the rapidity of the data collection, the minimal equipment requirements, and the simplicity

of training multiple individuals to use the method make it ideal for triaging and processing of remains.

**Lamendin Age Estimation Method, Mass Disaster, Forensic Identification**

## H50 Full Time Employment of Forensic Anthropologists in Medical Examiner's/Coroner's Offices in the United States—A History

*Hugh E. Berryman, PhD\*, Department Sociology & Anthropology, Middle Tennessee State University, Box 89, Murfreesboro, TN 37132*

After attending this presentation, attendees will have a better understanding of the development and expansion of forensic anthropology in the United States and its relationship with Medical Examiner's/Coroner's Offices.

This presentation will impact the forensic community by demonstrating a slow but steady increase in the demand for forensic anthropologists within the United States.

The second Luetgert murder trial in 1894 and the expert testimony of George A. Dorsey of the Columbia Field Museum in Chicago launched physical anthropology as a valuable judicial tool. Throughout the early and middle 20th Century, physical anthropologists employed in academic settings were increasingly called upon by law enforcement agencies to examine skeletal remains to ascertain identity. Wilton Krogman's 1939 publication in the FBI Bulletin entitled *A Guide to the Identification of Human Skeletal Material* stimulated interest in the medico-legal potential held by physical anthropology, and served as the earliest material evidence of a new specialty taking root. Krogman's expanded version of this bulletin appeared in 1962 under the title of T*he Human Skeleton in Forensic Medicine,* and provided a formal literary toolkit for early practitioners. In 1972, the American Academy of Forensic Science introduced Physical Anthropology as a new section and during the 1970's forensic anthropology courses began to appear on many campuses and graduates began to look beyond academia for employment. By 1980, physical anthropologists were being considered for employment in medical examiner and coroner offices, although their employers often struggled for justification considering the infrequent and sporadic nature of anthropology casework.

In September 1980, Hugh Berryman was employed as Director of the Shelby County Medical Examiner's Morgue in Memphis, Tennessee marking the first full-time employment of a forensic anthropologist in a medical examiner's office in the United States. The position of Morgue Director, with its administrative duties, provided justification for the position and insured the presence of a forensic anthropologist for the intermittent skeletal casework. In 1981, the New Jersey Medical Examiners Office hired Donna Fontana as full-time Forensic Anthropologist and Forensic Microscopist. She assisted with radiographs, identifications, and facial reconstruction. Currently, she has two interns and is the only anthropologist in the United States with direct access to a National Crime Information Center terminal.

In 1977, the Beverly Hills Supper Club disaster in Southgate, Kentucky claimed 165 lives and was the deadliest nightclub fire in United States history. This disaster provided the Kentucky Medical Examiner's Office the impetus to seek physical anthropological assistance from David Wolf between 1979 and 1980. From July 1980 to 1982, he worked under a personal service contract. In 1982, Wolf was employed full-time as Kentucky's and the nation's first State Forensic Anthropologist. In 1983, Berryman reorganized the Shelby County Morgue, eliminating two autopsy technician positions, and hired physical anthropologist Craig Lahren as Assistant Director to interface with the physicians and technicians, to bring an anthropology influence to the autopsy, and to facilitate research.

In 1984, William Rodriguez completed his pioneering work at the Anthropology Research Facility at The University of Tennessee, Knoxville and was employed as Deputy Chief Coroner at the Caddo and Bossier Parish Coroner's Office in Louisiana. In addition to forensic anthropology casework, he oversaw investigations including death scenes, and, as Deputy Chief Coroner, was responsible for psychiatric commitments. In 1986, Rodriguez left Louisiana for Syracuse, New York to become Forensic Anthropologist for the Onondaga County Medical Examiner's Office.

Also in 1986, Craig Lahren left Memphis opening the position of Assistant Morgue Director. The vacancy was immediately filled by Robert Mann who, after eight months, left for a position at the Smithsonian Institution in Washington, DC. Earlier that year, Steve Symes had been hired as Morgue Director for the Metropolitan Nashville/Davidson County Medical Examiner's Office only to leave that position late in 1986 to fill the Assistant Director's vacancy left by Robert Mann in Memphis. By 1987, Craig Lahren, who started this domino effect, became Coordinator of Forensic Services and forensic anthropologist for the Hamilton County Medical Examiner's Office in Chattanooga, Tennessee.

In 1979, when William Haglund began his career as one of thirteen Medical Investigators at the King County Medical Examiner's Office, Seattle, Washington, he was not an anthropologist. His duties required him to respond to all deaths occurring in his jurisdiction, collect evidence, interview families, perform death notifications and write reports. In 1983 he advanced to Chief Medical Investigator. However, it was the Green River Killings that began in 1982, and continued with 42 associated murders, that attracted him to forensic anthropology. With the completion of his Master's degree in physical anthropology in 1988, he added forensic anthropology to his duties as Chief Medical Investigator. After completing his PhD in physical anthropology in 1991, he worked four more years for the medical examiner's office before being employed in 1995 by the United Nations as Senior Forensic Advisor.

In 1989, William Rodriguez left Syracuse to accept a position as Chief Forensic Anthropologist and Deputy Chief Medical Examiner for Special Investigations for the Armed Forces Institute of Pathology, Washington, DC. The position of Kentucky State Forensic Anthropologist, left open by the untimely death of David Wolf in 1992, was filled in 1994 by Emily Craig.

For physical anthropologists, the 1980's marked the beginning of a shift in employment from academic to applied opportunities. That shift has continued and the numbers of forensic anthropologists employed full time in medical examiner's offices continues to increase, and their duties are more focused on their training. Austin and Fulginiti (2008) note that there are currently 19 forensic anthropologists hired in full-time positions with medical examiner's offices within the United States and an additional nine who are hired with shared duties. Encouragingly, Bradley Adams was hired as Forensic Anthropologist for New York City in 2004. He now has seven anthropologists that he directs—two with BA degrees, three with MA degrees, and two PhDs.

**Forensic Anthropology, Medical Examiner, History**

## H51 Death Investigation for Anthropologists: Examining an Alternative Role for Forensic Anthropologists in Medical Examiner's and Coroner's Offices

*Gina O. Hart, MA\*, Regional Medical Examiner's Office, 325 Norfolk Street, Newark, NJ 07103-2701*

After attending this presentation, attendees will learn how Forensic Anthropologists can use their training in alternative or dual roles at Coroner's or Medical Examiner's Offices and how Coroners and

Medical Examiners can benefit from having an anthropologist at their offices. Attendees will also learn what the typical role of the Medicolegal Death Investigator entails.

This presentation will impact the forensic community by demonstrating various ways that Forensic Anthropologists can use their training in alternative professions.

The purpose of this presentation is to demonstrate how forensic anthropologists can use their training in alternative or dual roles at coroner's or medical examiner's offices. Specifically, the author will focus on the typical role of a medicolegal death investigator, and illustrate what anthropologists should expect when entering into a career as a death investigator. Additionally, medical examiners will be informed how they can benefit from hiring forensic anthropologists in various roles in their offices.

The relationship between the pathologist and the forensic anthropologist has become vital in multiple medical examiner's and coroner's offices around the United States. These medical examiners and coroners have seen the added benefit of having a forensic anthropologist on staff in their offices, and have hired anthropologists to supplement their staffs. Unfortunately, medical examiners and coroners often have problems justifying a full-time anthropology position and have begun to hire forensic anthropologists in other areas within their offices.

Furthermore, the current popularity of forensic sciences in the media has caused students to enter forensic related programs at an increased rate. Therefore, the number of forensic anthropology students has reached an all time high while Anthropology positions at academic institutions have only slightly increased. As a result, current and future forensic anthropologists must investigate alternative career options if they wish to find employment in a related field. Medical examiner's offices offer several potential employment options for the anthropologist, including autopsy technician, compliance officer, photographer, and death investigator.

This presentation will focus specifically on the role of the medicolegal death investigator and how anthropologists can use their training in this role. The death investigator serves as the front line in medical examiner's and coroner's offices, investigating all cases that are reported and determining which ones will be accepted for examination. Tasks of medicolegal death investigators include performing scene examinations, collecting evidence that is directly related to the body, obtaining medical and social histories on decedents from family members and friends, and any other information that may help the pathologist to determine the cause and manner of death. Medicolegal death investigators come from a myriad of backgrounds, and they are expected to have a basic knowledge in medicine, medications, local legislation, and forensic sciences.

The experiences of working as a medicolegal death investigator and anthropologist at a medical examiner's office in a large urban area of New Jersey, covering four counties, are discussed. In 2007, 4,689 cases were reported and investigated by a 24-hour staff of 13 investigators. Of these cases, 1,825 were accepted and 1,249 were autopsied. From all the reported cases of 2007, manner of death included natural (3721), accidental (508), suicide (123), homicide (207), undetermined (30) and other types of cases (100). The other types of cases handled include non-human remains (18), cases transferred out of the jurisdiction (66), fetuses (14) and body parts (2).

In addition, the continuing education opportunities available to anthropologists who are interested in pursuing a career in medicolegal death investigation are discussed. A wide range of short courses are offered by numerous agencies throughout the United States providing the basic knowledge in Death Investigation. Training, publications, and other potential areas of continuing education will also be highlighted.

Anthropologists working as medicolegal death investigators have the unique experience of handling the typical death investigator cases and any anthropology cases from the initial stages of scene investigation.

This allows anthropologists a multi-dimensional perspective into the cases that they handle both as an investigator and a forensic anthropologist. Additionally, the medical examiner or coroner will greatly benefit by having a forensic anthropologist on staff, even if they are not able to justify having that person in a full-time forensic anthropology position.

**Medicolegal Death Investigation, Forensic Anthropology, Medical Examiner's Office**

## H52    Identification of Multiple Cranial Traumas in a Recently Closed Homicide Investigation

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After attending this presentation, attendees will come away with an understanding of the value of using forensic anthropologists in investigative contexts involving human skeletal remains. This will be achieved through an overview of a recently resolved homicide case from southeastern Wyoming that contrasts and evaluates the assessments offered to law enforcement by forensic anthropologists and other forensic investigators involved in the case.

This presentation will impact the forensic community by providing a direct illustration of the power of employing forensic anthropological techniques in crime investigations, in service to law enforcement as a means for remains identification and for analysis of human skeletal trauma.

Attendees will be presented with a case study of an actual homicide investigation involving human skeletal remains. This case serves as an example of the benefits of applying forensic anthropological techniques to the identification of remains and in analyses of bone trauma. In the initial stages of this investigation, which began in December 2002, both anthropologists and forensic pathologists were approached and engaged by law enforcement to provide analyses of an unidentified, partial human skeleton. This set of remains, found through extensive, coordinated searches conducted over a period of several months in 2002 and 2003 by volunteers and multiple law enforcement agencies and rescue and recovery groups, consisted of a partial cranium, both femora, a humerus, and an ulna. These skeletal elements were scattered over an area of roughly one square mile on the ground surface in a remote recreational area. The authors will present the osteological evidence available to investigators at the outset of this homicide investigation, and will detail the course and outcome of the case. The specific evidence emphasized will be multiple traumas to the recovered portion of the cranium of the victim, including a unique penetrating fracture to the occipital region, and several other perimortem traumas to the mid-facial and orbital regions of the skull. The hypotheses offered to law enforcement by anthropologists and pathologists differed markedly in terms of the suggested mechanisms of injury and in the suggested relationships, both temporal and physical, of the observed cranial traumas.

Subsequent case evidence and testimony provided investigators with detailed information regarding the true mechanisms of the various injuries, as well as the relationships between these observed and documented traumas. This information provides a unique opportunity to present both a comparison of the hypotheses offered by the anthropologists and other forensic investigators to the actual chain of events for this homicide, and an evaluation of the accuracy of these hypotheses, which were based solely on the initial osteological evidence. It will be shown that the hypotheses offered to law enforcement by the

case forensic anthropologists correlate most closely with the actual mechanisms and sequence of traumas for this homicide case, which was closed in early part of 2008. This will be made explicit through the presentation of the information contained in the documented statements provided to law enforcement by the perpetrators of the crime.

**Osteology, Homicide, Anthropology**

## H53 Anthropologist/Medical Examiner Collaboration at Isolated, Inaccessible, or Disrupted Crime Scenes

*Emily A. Craig, PhD\*, Medical Examiner's Office, 100 Sower Boulevard, Suite 202, Frankfort, KY 40601*

The goal of this presentation is to demonstrate some cases of unusual and difficult body recovery scenarios wherein the on-site efforts of the forensic anthropologist helped the consulting medical examiner correlate autopsy findings with the death scene. The interplay of innovative and traditional techniques will be discussed as well as the inseparable disciplines of forensic anthropology, musculoskeletal anatomy, and anatomical pathology.

This presentation will impact the forensic community by presenting one aspect of the symbiotic relationship between forensic anthropology and forensic pathology.

The Commonwealth of Kentucky combines a coroner system with a single state-wide medical examiner system which is under the ultimate control of the Justice and Public Safety Cabinet within the State Government system. There is an elected coroner in each of Kentucky's 120 counties and there are four separate medical examiner offices, all under the direction of a chief medical examiner. By statute (KRS 72) the state provides medical examiner services to all county coroners, and this provision includes the services of the state's forensic anthropologist. The forensic anthropologist is employed full–time within the medical examiner's office and has dedicated laboratory and office space in the Centralized Laboratory Facility in Frankfort, Kentucky. This anthropologist is on call for response to any of the county coroners for on-site investigations and will demonstrate how the timely arrival at the crime scene and almost simultaneous integration of information to and from the consulting pathologists proves to be advantageous.

It almost goes without saying that each death scene needs thorough documentation and analysis in order to explain some of the disarray and damage seen at autopsy, but it is the anthropologist's on-site analysis on cases with varying degrees of disruption and/or decay (not just skeletal remains) that has helped integrate information from the scene and autopsy. The number of disrupted human remains in each jurisdiction is probably not out of proportion to the overall number of autopsies performed each year (3.5 to 4%) but because of the geography of the state, the range of weather conditions, and varied carnivore predation patterns, on-site analysis by a forensic anthropologist proves especially helpful in cases wherein any combination of taphonomic events may have modified the death scene. Although the primary focus for anthropologist's crime-scene response is skeletal remains, a thorough knowledge of overall soft tissue gross anatomy in addition to osteology has proved to be invaluable for the documentation and collection of evidence with any disrupted remains, regardless of the amount of decay. A working knowledge of entomology, botany, and wildlife biology has also been necessary to incorporate associated evidence into the overall death investigation.

The cases to be discussed in this presentation are those in which the forensic anthropologist assisted with the scene recovery and site analysis while in contact with a morgue-based medical examiner, thus demonstrating the symbiotic relationship between the two. In addition to standard image transfer through phone systems, some of the other scene-to-morgue procedures used in Kentucky involve hand held 3-D microscopes with internet connections and radiographic identification capabilities through mobile data terminals.

Fire-related deaths also comprise a number of the cases to be discussed; from an underwater structure recovery to incidents with multiple homicides disguised by fire. Since the percentage of overall homicides disguised by fire has reached as high as 21% per year, there remains a high level of suspicion in nearly all fire-related deaths across the Commonwealth. Here, as with any skeletonized remains, anthropological recovery techniques help preserve evidence. Therefore the anthropologist's on-site protocol ensures a thorough transfer of information *and* remains to the pathologist.

**Anthropology, Medical Examiner, Crime Scene Investigation**

## H54 The Role of Forensic Anthropology in Disaster Operations

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After attending this presentation, attendees will understand the growing role of forensic anthropologists in disaster operations within the medical examiner system.

This presentation will impact the forensic community by demonstrating the growing role of anthropology and the additional training necessary in being a part of disaster operations. The diverse educational and professional backgrounds of anthropologists make them strong candidates for taking on roles that may seem less traditional for anthropologists in this setting.

The skills of forensic anthropology are essential components in the normal operations within the medical examiner or coroner's office. More recently, however, the importance of integrating anthropology in disaster operations has been noted. Such an instance was exemplified following the renewed recovery efforts of the World Trade Center site in 2006 in which the Office of Chief Medical Examiner-New York City (OCME-NYC) recognized the critical role of anthropology for these types of operations. The OCME-NYC is spending considerable time and resources in developing operational teams that specialize in recovering, processing, analyzing, identifying and managing remains from mass disasters. The goal of this presentation is to discuss the multi-level operations and highlight the role of forensic anthropology in each area of fatality management.

**Field Operations:** The recovery of human remains following an incident is a multi-agency effort that, depending on the nature of the disaster, involves forensic anthropologists working in different capacities. The OCME-NYC has established a forensic anthropologist in the role of the Fatality Management Branch Director within the National Incident Management System – Incident Command Structure. This role is a lead player within the operations section and to relay scene information to the OCME Incident Commander, Investigative and Recovery Teams, and to other city agencies involved in supporting fatality management. The more common role of the anthropologist in field operations involves the application of archaeological methods (i.e., survey, mapping, excavation). It is in this role that training in archaeology is the essential component for ensuring that the most appropriate, comprehensive and accurate recovery techniques are utilized during this stage of the operation. Due to the potential hazards in mass disaster incidences, recovery operations might need to be conducted under HAZMAT conditions. Forensic anthropologists involved in these operations must have specialized training to work under these conditions and be familiar with appropriate personal protective equipment. Furthermore, the anthropologist must be able to

recognize potential hazardous materials in order to alert specialists that characterize and mitigate such hazards. At the OCME-NYC, forensic anthropologists have also been integral part of developing specialized equipment for disaster field operations. For example, the OCME has developed a mobile sifting platform to expedite the recovery of remains from large volumes of soil, while maximizing forensic integrity.

**Morgue Operations:** Following recovery operations, remains are accessioned into the disaster morgue. Before the remains can be processed through the various stations of the morgue they are triaged. In the case of fragmented or commingled remains, anthropologists are well-skilled to associate or segregate remains and establish appropriate case numbers. The anthropological analysis station within the disaster morgue is where the typical role the forensic anthropologist is instituted. The anthropologist works along side the medical examiner to develop biological profiles, conjoin fragmented bone, and assist with trauma analysis. The anthropologist may also assist Forensic Biology in making hard tissue selections for DNA analysis.

**Disaster Victim Identification (DVI) operations:** The DVI process reconciles postmortem data from the recovered remains with antemortem data related to the victims. DVI at the OCME-NYC is operated by the Forensic Anthropology Unit with an anthropologist in the position of DVI Manager. The role of the DVI Manager is to review the collective work of the identification teams (e.g., DNA, odontology, fingerprints) and oversee the identification process. The DVI Manager also coordinates the Identification Review Board, which provides the final recommendation on all identifications. Anthropologists on the DVI staff may also be present in the morgue and the Family Assistance Center. Additionally, the DVI group has played a major role in the development of the Unified Victim Identification System (UVIS), a program developed at the OCME-NYC that allows access to all data related to an incident.

The diverse educational and professional backgrounds of anthropologists make them excellent choices for work associated with disaster operations. The skills, knowledge, and training of personnel within New York City's Forensic Anthropology Unit play an integral role in the City's mass fatality response plan.

**Mass Disaster, Disaster Operations, Anthropology**

## H55 The Forensic Anthropologist, the National Crime Information Center (N.C.I.C.), and National Missing and Unidentified Persons\System (NamUs) Databases

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After attending this presentation, attendees will understand how the forensic anthropologist can be an active and effective participant in utilizing the national informational databases in the investigation of unidentified persons.

This presentation will impact the forensic community by highlighting the key points of the National Crime Information Center (N.C.I.C.) and National Missing and Unidentified Persons System (NamUs) databases, and the challenges faced by the forensic anthropologist.

In this age of instant communication, public opinion expects investigations to be completed in a 60-minute time frame. Some of the primary tools used by law enforcement for gathering and organizing the immense amount of information and scientific data collected in the course of an unidentified person investigation are the N.C.I.C. and NamUs databases.

N.C.I.C., maintained by the F.B.I., is a nationwide online computer/telecommunication system, which contains millions of records of persons and property records from across the United States. N.C.I.C. was established in 1967 and at the current time is used by all 50 states, Puerto Rico, U.S. Virgin Islands, Guam, Canada, and federal agencies. It is accessible only by criminal justice and law enforcement agencies. Medical examiner/coroner agencies can have access if they meet certain criteria set through shared management between FBI and state and local law enforcement.

In unidentified person investigations, N.C.I.C. captures identifying information such as personal descriptors and dental data, which is entered by the investigating law enforcement agency. N.C.I.C. will automatically cross-match this information against missing and wanted persons' records. This comparison is performed on a daily basis on the records that have been entered or modified the previous day. A list of potential matches is generated for each agency that enters information. These entries and matches are restricted to the agencies that have access. Verification of scientific data entered on unidentified person cases by the forensic science community is usually not routine.

The medical examiner/coroner, or their designee, controls the case information entered or edited into the NamUs unidentified database, after an online registration. Unlike N.C.I.C., NamUs is web-based, though not all uploaded information on the case is viewable by the public. Personal descriptors and dental information are entered into the NamUs database, after review by a member of the forensic science community, such as the forensic anthropologist. NamUs has strived to simplify its entries, making it user-friendly to the public. Active participation by the forensic anthropologist, medical examiner, or forensic odontologist in the entry, modification, and comparison of unidentified person data ensures that accurate information is entered into the database. This process is imperative in the comparison with missing persons' information.

This presentation will demonstrate how the active participation by the forensic anthropologist can ensure accuracy of database information, which may increase the number of individuals who could be identified. It is recommended that those in the forensic science community working with unidentified persons become an active participant with the national databases used in these investigations.

**Forensic Anthropology, N.C.I.C., NamUs**

## H56 The Role of the Harris County Medical Examiner's Office Forensic Anthropology Division in Scientific Identification

*Jason M. Wiersema, PhD\*, Harris County Medical Examiner, Anthropology Division, Houston, TX 77054; and Jennifer C. Love, PhD, Sharon M. Derrick, PhD, and Luis A. Sanchez, MD, Harris County, Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will understand the role of the Harris County Medical Examiner's Office Forensic Anthropology Division in scientific identification.

This presentation will impact the forensic community by outlining the contribution made by Forensic Anthropology to the scientific identification of unknown and tentatively identified decedents.

The Harris County Medical Examiner's Office (HCMEO) Forensic Anthropology Division (FAD) is a relatively new division, established in November of 2006. Scientific identification of unknown and tentatively identified decedents is one of the core responsibilities of the Medical Examiner's Office and has become a central component of the daily activities of the FAD.

HCMEO standard operating procedures require scientific confirmation of identification of all homicide cases and all cases

rendered unsuitable for visual identification by decomposition, disfigurement and thermal injury.

All cases in which scientific identification is not confirmed by fingerprints or dental comparison become the responsibility of the FAD. When available, the FAD scientifically confirms identification through radiograph comparison and facilitates identification through DNA by collecting family reference samples and interpreting DNA kinship indices. When antemortem records or DNA samples are unavailable, the FAD facilitates the identification through the collection and presentation of circumstantial evidence to the responsible pathologist. In the case of unknown decedents, the FAD compiles an unknown decedent description; disseminates it to law enforcement, media, and the Unidentified Decedent Reporting System; submits DNA to CODIS; and follows up on all generated leads.

Since its inception, the FAD has significantly reduced the number of cases that remain unidentified. The number of decedents who remain unknown for greater than six months decreased 40% from the three-year average prior to the inception of the FAD to 2007 (the first full year of operation). This number decreased substantially (90%) again in the first half of 2008. The success is a result of a dedicated team of professionals that can focus on the follow-through with law enforcement, media, and the community to identify the unknown and to notify the next-of-kin. Furthermore, an audit of all of the unknown decedent case files that predated the FAD has yielded identifications of 30 previously unknown decedents.

Anthropologists have significantly reduced HCMEO DNA costs. Prior to the FAD, cases without dental records were scientifically identified through DNA. A majority of these cases are now identified through skeletal radiograph comparison. The FAD completed a total of 31 scientific identifications in its first full year of operation, 2007, and had completed 22 during the first half of 2008. Identifications are routinely performed using an array of radiograph types.

Fourteen (45%) of the 31 identifications in 2007 were made based on chest radiographs. Five comparisons were made between lumbar spine images, and the remainder of the 2007 cases involved skull, hip, knee and wrist images. Thirteen (62%) of the 21 2008 cases for the first half of 2008 involved chest radiograph comparison, and the remaining eight were completed with head, pelvis, foot, wrist and shoulder films. Each of these identifications was completed at the request of the responsible pathologist.

Another beneficial by-product of the increased efficiency in decedent identification has to do with morgue capacity. The increase in efficiency necessitates the curation of fewer remains and a shorter curation period for unidentified decedents. This represents a considerable benefit to the HCMEO's disaster preparedness by increasing on-site surge storage capacity.
**Forensic Anthropology, Scientific Identification, Medical Examiner's Office**

## H57  Forensic Anthropology at the Pima County (Arizona) Office of the Medical Examiner: The Identification of Foreign Nationals

*Bruce E. Anderson, PhD\*, and Walter H. Birkby, PhD, Forensic Science Center, 2825 East District Street, Tucson, AZ 85714*

After attending this presentation, attendees will gain an understanding of some of the issues involved in the identification process for a large number of foreign nationals.

This presentation will impact the forensic community by sharing lessons learned through the identifications of hundreds of foreign nationals over the past eight years.

Over the past eight years, the caseload of the forensic anthropologists at the Pima County (Arizona) Office of the Medical

Examiner (PCOME) has changed considerably due to the dramatic increase in the number of migrants who have perished in the Sonoran Desert while attempting a clandestine entry into the United States. The last eight years have witnessed greater than a ten-fold annual increase in the number of these deaths and required forensic anthropological examinations for nearly half of the 1100 migrant cases through 2007. Approximately one-quarter of all of the anthropological examinations within our office during 2000 involved migrants, but by 2007 more than three-quarters of our exams were on these foreign national cases. This increase in the annual casework involving foreign nationals takes on greater importance when Tucson's rapidly-growing metropolitan area is considered. Add to this the fact that the PCOME provides medicolegal examinations for ten of the fifteen counties in Arizona, including one of the fastest growing counties (Pinal) in the U.S., and one begins to appreciate that the two forensic anthropologists employed at the PCOME have more than enough work to do. The analyses of cremains, personal identification through radiographic comparisons, trauma analyses, the generation of biological profiles, and legal testimony are some of the varied duties that the forensic anthropologist performs on the non-migrant cases. However, the migrant cases have required more effort, on average, and not merely because of their greater number but also because of the challenges encountered in attempting to effect an identification. Adequate antemortem records are non-existent in many instances, at times necessitating that the generated biological profile must be the primary means of comparison to family recollections and reconstructed antemortem records. These circumstantial identifications that are partially based on the anthropological results are not ideal but necessary given the constraints of time and funding. DNA analyses have been utilized in more than sixty cases over the past seven years, but the cost and the wait have at times proved prohibitive. Even utilizing circumstantial identifications, our overall rate of identification still hovers around 70%. On the plus side, this rate may be expected to increase as DNA databases are created that compare sequences or profiles from postmortem samples of unknowns to those of family reference samples from missing migrants. Another plus is that much has been learned from the anthropological research conducted on many of these foreign nationals, whose ancestry appears to be overwhelmingly Southwest Hispanic (defined here as ancestry relating to Mexico and Central America, and excluding the Caribbean). Researchers have collected data on more than 500 of these individuals, with slightly less than half being identified. Data collected include craniometrics, infracranial metrics, cranial discrete traits, stature, dental health, and mtDNA sequences. By analyzing the skeletons of more than 200 identified foreign national Southwest Hispanics over the past eight years, our office is now better able to characterize the more than 300 unidentified presumed migrants as likely being Hispanic. Forensic anthropologists around the U.S. may benefit from this increase in knowledge because Hispanics, from both Meso-America and the circum-Caribbean, can be expected to continue migrating throughout the lower 48 states. Undoubtedly, some of these individuals will be encountered in future forensic anthropological examinations.
**Forensic Anthropology, Foreign Nationals, Identification**

## H58  Forensic Pathology and Anthropology: A Collaborative Effort

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After attending this presentation, attendees will understand the diverse roles served by the Harris County Medical Examiner's Office Forensic Anthropology Division and the spectrum of trauma casework the division receives.

This presentation will impact the forensic community by introducing students and academically based forensic anthropologists to the roles served by forensic anthropologists working within the medical examiner's office and the versatility of forensic anthropology to assist medical examiners in the determination of cause and manner of death.

The Harris County Medical Examiner's Office Forensic Anthropology Division (FAD) is a relatively new division, established in November of 2006. Since the division's inception, the FAD has developed into a three-pronged service: Skeletal Recovery Team (SRT), Identification Unit (ID Unit), and Forensic Anthropology Services (FAS). As the SRT, anthropologists attend crime scenes requiring specialized skills for processing such as scenes of skeletal, burned, dismembered, commingled and buried remains. As the ID Unit, anthropologists create and disseminate unidentified decedent descriptions; facilitate identification on difficult cases through skeletal radiograph comparison, interpretation of DNA kinship indices, and collection and review of circumstantial evidence; and track and refer decedents for county burial. Through the FAS, the anthropologists serve as consultants on medicolegal cases by developing skeletal profiles, interpreting bone trauma, and assessing bone pathology. Although the skills required to serve in all three roles are based in the fundamentals of physical anthropology, each role requires flexibility and adaptability. The purpose of this presentation is to demonstrate the diverse role taken by forensic anthropologists at the Harris County Medical Examiner's Office (HCMEO) through an overview of trauma cases received by the FAS.

Prior to the development of the FAD, the HCMEO outsourced skeletal remains for analysis to academically based forensic anthropologists. Previous to outsourcing, skeletal remains were analyzed by forensic pathologists and either released for burial or archived in the morgue. Consultation was not typically sought for bone trauma analysis. When the FAS was established, the first task was to reexamine skeletal remains archived at the HCMEO. These remains represented unidentified decedents and required skeletal profile development and trauma analysis. In addition to the archived remains, the FAD received 154 cases in 2007, the first complete year the division was in operation. Of these incoming cases, only nine required the development of a skeletal profile. One hundred and forty-eight cases required trauma analysis and/or skeletal radiograph comparison. The breakdown of these cases is as follows: 6 non-human or non-forensic, 30 skeletal radiograph comparisons, 11 bone pathology, 42 blunt force trauma, 16 child abuse, 33 sharp force trauma (impression evidence analysis), 9 ballistic trauma, and 1 combined sharp and blunt force trauma. Nine of the 154 cases involved thermal trauma.

Analysis of trauma and bone pathology occurs either *in situ* or by removing and processing elements for more detailed analysis. Determination of the necessity of an anthropologic consultation takes place in the autopsy suite. Joint discussion regarding the case and the finding(s) in question between the anthropologist and the pathologist determine whether a complete anthropologic consultation with removal of skeletal elements is warranted. This joint determination has proved invaluable on numerous occasions. Techniques utilized for the analysis include gross documentation and inspection, examination under a dissecting microscope, and when necessary, casting for tool mark interpretation.

The interpretation of trauma often contributes to the reconstruction of events surrounding death, classification of manner of death, and ultimately, the adjudication of the case. For example, differentiating between a simple fracture pattern associated with a fall and a complex fracture pattern associated with multiple impacts may add pivotal support for case classification: homicide vs. accident. With child abuse cases, recognizing remote and acute injury, interpreting fracture distribution and aging fractures can reveal a pattern of abuse as well as a timeline of traumatic episodes, all critical elements in the prosecution of the case. Evaluation of rib fractures has been invaluable in the documentation of a new artifact of resuscitation related to the use of an automated chest compression device. Interpreting impression evidence can provide a description of a weapon's cutting edge, enabling exclusion or inclusion of a possible suspect weapon.

Cases that require anthropologic consultation range from the obvious, such as skeletal remains or abused children to the innocuous, such as an alcoholic homeless man found dead near his makeshift residence. The make-up of the casework received by the FAS exemplifies the knowledge base a forensic anthropologist must have to operate in a medical examiner's office. A strong working knowledge of bone biomechanics, impression evidence interpretation, and bone pathology and healing is as important as aptitude in standard methods to estimate age, ancestry, sex, and stature of skeletal remains. Fiscal restraints may inhibit medical examiner's offices from securing a full-time anthropology position; however, the variety of skills an anthropologist possesses to assist with the entire death investigation process should justify the expenditure.

**Forensic Anthropology, Medical Examiner's Office, Trauma Analysis**

## H59 Maintaining Custody: A Virtual Method of Creating Accurate Reproductions of Skeletal Remains for Facial Approximation

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After attending this presentation, attendees will gain insight into new computerized methodologies that will assist local law enforcement and forensic facial approximation specialists by providing the results of a unique three-dimensional pilot study conducted by a team of researchers.

This presentation will impact the forensic community by serving to increase scientific knowledge of new technologies and methods available to the forensic community for human cranial identification. It will also attempt to advance the state of facial approximation methods which are commonly utilized by law enforcement to identify unknown individuals.

Facial approximation is a common analysis requested by local and national law enforcement agencies for human identification. Evidentiary chain of custody concerns by law enforcement have led agencies to search for technological alternative methods that can allow the agency to maintain custody of critical evidence while getting cutting edge forensic analyses. Three-dimensional imaging technologies allow researchers to go beyond traditional anthropological methods to now create virtual computed models of anatomical structures.[1] The goal of this project was to provide accurate skull models to forensic identification specialists and Medical Examiner's Offices for forensic facial approximation without taking custody of the skeletal material itself.

In this pilot study, unidentified skulls were taken by several Medical Examiner's Offices in the state of Florida to local radiology centers where the skulls were scanned on a 64-slice high resolution computed tomography (CT) scanner at a slice thickness of 0.5mm. The CT data was then given to the researchers to compute anatomically accurate virtual models of the skulls. It should be noted that the researchers never took custody of the remains and in many cases never had physical contact with the remains at any time. The volumetric data from the scans were taken into the visualization software package *Mimics*© version 12 (Materialise). A FloodFill method of seeding the image was done to model the bone pixels in the data set. This 3-D volumetric pixel grouping was then filtered of artifact holes and closed to create one

unsegmented structure. The data set was then rendered into a 3-D Model and exported as a Stereolithographic file (STL). After a biological profile was created from the virtual skull data by a forensic anthropologist,[1] the STL models were exported for rapid prototyping. Accurate full size prototypes of the skulls were then produced from the computed virtual skull model using a 3-D ZPrinter 310 (© ZCorp) printer which was then submitted to the forensic facial clay modeling specialist.[2]

The clay modeling specialist and anthropologist worked with the researchers on highlighting key facial regions used in facial reconstruction that are critical in approximating the soft tissue features. From their input, focus was turned to regions such as the vomer for the estimation of the nose shape and length and suture lines for the estimation of age. Due to the lack of objective criteria for analyzing the facial approximations themselves other than superimposition, the researchers are currently focusing on developing methodologies to interpolate the soft tissue relationships from the surface voxels on the computed representation of the skulls.

This project demonstrates the potential for high-end forensic analysis to be conducted remotely without assuming custody of the evidence. This also allows local law enforcement agencies to have access to experts beyond their geographic location. While there is a wide range of variation between commonly used facial identification methods,[2] the benefit of this study is that there is an open discussion of the strengths and weaknesses at each stage of the analysis which will in turn increase the understanding and scope of forensic facial approximation from 3-D data.

**References:**
[1]   Decker SJ, Hilbelink DR, Hoegstrom EJ. Virtual skull anatomy: three-dimensional computer modeling and measurement of human cranial anatomy. Proceedings for the 60th Annual AAFS Meeting, Washington, DC 18-23 Feb. 2008; (14)312.
[2]   Decker, SJ, Hilbelink DR, et al. Who is this person? A Comparison Study of Current 3-Dimensional Facial     Approximation Methods. Proceedings for the 60th Annual AAFS Meeting, Washington, DC 18-23 Feb. 2008; (14)338.

**3-D Modeling, Evidence, Human Identification**

## H60   The Role of Adult Age-Related Craniofacial Changes and the MORPH Database in Computer Automated Face Recognition Research and Development

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The goal of this presentation is to present how osteological and anatomical knowledge, in terms of adult age-related craniofacial morphologic changes, are used in computer science research for forensic purposes.

This presentation will impact the forensic community by explaining the key ways in which craniofacial morphologic changes occur across the adult age span and will provide information of the relevance of this information to computer automated face recognition technology through a discussion of how these data are currently being used.

First, attendees will gain an understanding of the key ways in which craniofacial morphologic changes occur across the adult age span, in terms of the general sequence and pattern of change due to normal aging effects. Secondly, attendees will be informed of the relevance of the above information to computer automated face recognition technology through a communication of how these data are currently being used, namely through research conducted using the MORPH database.

Further, attendees will be updated on the status of the dynamic MORPH database, founded in 2003, and currently expanding. Recently, the MORPH database has had a significant impact internationally as well as domestically, mainly within the computer science arena, but its existence still remains largely unknown to many practitioners and researchers within the forensic anthropology community, and the forensic science community at large. This presentation will help disseminate features of this ongoing work.

An awareness and understanding of the application of knowledge stemming from studies related to craniofacial age changes, bone remodeling of the skull, and so forth, has been raised within the computer science community. In turn, an awareness of the ways in which the computer science community is using osteological and anatomical information of this nature is critical for those forensic anthropologists who might presently, or in the future, desire to conduct individual or collaborative research involving adult age-related craniofacial remodeling and or forensic face recognition/facial reconstruction methods and technologies.

For example, within the security and law enforcement venues, face recognition research typically involves testing the efficacy of computer algorithms to match later-obtained digitized images of individuals' faces (usually perpetrators of crime, terrorists, missing persons, or fugitives) with existing or earlier-obtained images of the same individuals (digitized mug shots, photographs, and the like). A further step in research of this nature has been to explore the capabilities of computer algorithms to match faces after a significant period of time has passed between facial images, or rather, after a number of years have passed, after the individual in question has aged, five, ten, twenty, or more years. This is where understanding the sequence, pattern, and variation of age-related craniofacial morphologic changes comes into play.

A synopsis of the ways in which the computer science community is using information and about how the craniofacial complex remodels during the adult decades of life will be presented, followed by an overview of the current status of the MORPH database. The MORPH database comprises thousands of images of thousands of individuals and has played a vital role in our understanding of face recognition and how faces age from late adolescence through senescence, given that large sample sizes of known faces aged across the adult lifespan have now become available for study.

**Age-Related Craniofacial Remodeling, Computer Automated Face Recognition, MORPH Database**

## H61   Skull/ Photo Superimposition Validation Study

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The goal of this presentation is to demonstrate a unique method of validation using skull models from living subjects.

This presentation will impact the forensic community by illustrating how advances in technology can provide new validation methods from living subjects.

The mission of the Joint POW/MIA Accounting Command Central Identification Laboratory (CIL) in Hawaii is to search for, recover, and identify missing U.S. service personnel from past wars.

Difficulties in identification present themselves when the skeletal remains are of similar biological profiles, commingled, and when mtDNA testing is not possible due to poor preservation of the osseous remains or the lack of comparative family reference samples (buccal swabs or blood drop cards). A quantitative Cranial/Photograph Superimposition technique has been developed at the CIL to support

vetted identification methods (anthropological analysis, dental record and historical record comparisons) and as a means of probable exclusion when inconsistencies in congruity can not be explained. The quantitative Cranial/Photograph Superimposition incorporates a line-up of photographs for blind analysis comparison to the crania. Using video-camera overlays, each photo is aligned to the 'best possible fit' by resizing each photo and adjusting/aligning the skull to fit with the subject's facial orientation as depicted in the photo. Alignment criteria scoring is used to rate the congruity of ten features of the superimpositions. Each feature is scored as a +1 (good fit), -1 (lack of alignment), or 0 (for areas not seen in the photograph or if there is trauma to the skeletal remains). This scoring procedure results in a final comparison sum, with a maximum of ten points for each photographic and skull comparison.

As with all new methods, validation is crucial in determining the accuracy and scope of use, and requires a known-error rate of the method or procedure based on known comparatives (crania). This research will demonstrate the use of CAT scan data to generate precise 3-D copies ("models") of skulls of known individuals (living CIL staff members) that are then used in a comparative study of cranial alignment using skulls and photographs as developed at the CIL. Ten current and former CIL employees with like gender and ethnic affiliation were chosen for the known sample group. All subjects were CAT scanned using the lowest slice rate possible in order to optimize the detail of their cranial features. The raw x-y-z data from each CAT scan were converted to a 3-D print file. Three dimensional replicas of the ten skulls were produced, cleaned, infused with a hardening agent, and assigned bar codes (for concealing the skull identities). Scoring sheets with matching bar codes were provided to the test participants for each skull's comparison.

CIL summer interns and new employees were chosen to perform the validation study. All participants were individually taught the CIL's quantitative scoring, blind photo line-up procedure, and were given an opportunity to practice the method on various non-study related material prior to beginning the validation study. The participants aligned, compared, and scored ten photographs with each skull.

Preliminary results indicated that intern examinees at the CIL selected the correct photograph in 40% of cases examined (i.e., found no inconsistencies between the corresponding/actual photograph and skull) and eliminated all other photographs in the blind "line up." The false positive rate in this study was 60%. Additional work by researchers beyond the novice level and with varying experience is necessary before drawing final conclusions on the accuracy or utility of photo superimposition.

**Superimposition, 3-D Models, Validation Technique**

## H62 The Importance of Morphological Traits in Facial Identification

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The goal of this presentation is to show the first results of a research project concerning visual identification for forensic purposes funded by the European Union (AGIS 2005) and involving the University of Dusseldorf, Milano and Vilnius.

This presentation will impact the forensic community by exposing the importance of facial morphological traits for personal identification.

Identification of the living is a complex procedure which is becoming more and more requested by judicial authorities (e.g., for individuals observed on video while they are perpetrating a crime). Forensic scientists are therefore frequently requested to identify faces, for example, to tell if a suspect can be identified from a picture based on facial morphology and characteristics. Literature shows that the use of facial indices may be extremely dangerous and lead to erroneous identifications, whereas the potential of simple facial morphology, for which several classifications exist (Interpol, Vanezis, Assmann et al.), has never been studied in depth. This is mainly because no large scale study on the distribution of specific facial morphological traits has been previously performed.

The initial task was to verify the distribution and frequency among a European male population of about 1,000 individuals (20-30 years of age) of 43 specific facial traits (e.g., facial form, nose tip shape, philtrum shape, head shape, frontal height, eyebrow edge, lid axis, nasal root, labial breadth, etc. as in the Assmann et al. classification which contemplates a larger number of characteristics), and observe their discriminant value and inter and intra observer error. A group of over 900 faces was photographed and then the traits were classified with the Assmann atlas by recording the different shape of every one of the 43 relevant facial characteristics. This process was completed twice by the same observer and every face was also classified by two different observers, a layman and an expert. Results have shown the homogeneity of facial traits among the German, Italian and Lithuanian population, especially the head and chin shape, whereas the frontal and nasal characteristics seem to present larger variation. Classification of the results showed low interobserver and intraobserver errors for some traits but fairly large for others, for example the evaluation of chin and facial shape. A mean of 39% interobserver and 30% intraobserver error was recorded. Moreover, the high inter-individual variability was highlighted, with significant differences between analyses from the same individual and between laymen and experts. Although slight differences can be observed between the judgments from the same observer, the most relevant discordances were recorded between laymen and experts. These results highlight the importance of experience in analysis and interpretation of morphological traits. This study also shows some of the pitfalls concerning the mechanisms of facial identification through morphological facial traits. Although there is no common standardization, the use of references helps in the description of morphological traits. The description and judgment concerning the specific shape and appearance of each facial trait; however, remains a subjective process which may present significant differences between different observers.

The results have shown an interesting scenario of the respective frequencies of different facial traits which may be useful for identification. Nonetheless, the high subjectivity in face evaluation requires caution in considering morphological classification as a crucial tool in facial identification. These results, therefore, suggest a prudent approach to visual identification where classification of facial morphology may be useful in narrowing down a list of possible matches, but cannot be a model to prove identity. Identity should be proven, when possible, by implementing general morphological data with a more detailed analysis of facial forms, for example by procedures such as superimposition.

**Forensic Anthropology, Personal Identification, Facial Identification**

# H63 Fingering a Murderer: A Successful Anthropological and Radiological Collaboration

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After attending this presentation, attendees will appreciate the validity of small, but unique, trabecular patterns in bones or bone fragments for purposes of positive identification of unknown burned remains.

This presentation will impact the forensic community by emphasizing the value of radiological evaluation of incinerated skeletal fragments where DNA extraction is impossible, and the meticulous anthropological work essential to provide identifiable specimens for radiological study.

A 24-year-old man reported missing by his mother was last seen on a rural "horse farm" in New Hampshire in the company of the somewhat reclusive female owner. She had a history of multiple tumultuous relationships and a previous assault charge. A warrant was obtained to search the property. Burned bones, a burned mattress, a burn-barrel and other burn sites were eventually discovered, yielding 30 five-gallon buckets-full of burned material. After initial screening by the New Hampshire Medical Examiner's Office, several hundred burned bone fragments and other artifacts were sent for analysis to a physical anthropology team in an adjoining state.

Several hundred hours were consumed in separating and photographing human bones and teeth fragments from those of at least eight non-human species. There was evidence that there had been intentional mixing and scattering of remains between the several burn sites. This substantially increased the difficulty of the anthropological task. When feasible, the fragments were identified as to anatomic site and laterality, individually numbered and labeled, packaged in small plastic specimen bags for DNA-protocol handling, individually boxed and labeled, then sorted for analysis by odontology, radiology, and mitochondrial DNA.

Anthropological examination produced the biological profile of a male, in his early 20s, of indeterminate stature and ancestry, with no evidence of pathology or perimortem trauma. The odontologist found the dental remains to be consistent with the presumed victim's age and previous dental radiographs, but insufficient for positive matching. Least burned fragments of bone and teeth were sent for mitochondrial DNA testing, but too much organic material had been lost to produce a signal.

Antemortem radiographs of the missing person were obtained including images of the dental arches, facial bones and lower half of the skull, the right shoulder, the lumbosacral spine and the left hand and wrist. All were normal. These were sent along with the boxed and labeled fragments for radiological evaluation. Each specimen was visually compared with the antemortem images. Promising fragments were painstakingly positioned to replicate the projection of that part on the antemortem study. Since the victim had been young and healthy, there were no skeletal features of disease, degeneration, tumor or trauma. Therefore, comparison was limited to the external configuration and internal trabecular pattern of each specimen. Most of the fragments were devoid of even these features when radiographed with fine detail using a mammography x-ray unit and film. After multiple re-positioning and re-examination of fragments, positive matches were found in unique trabecular patterns in the terminal phalanges of the index and ring fingers and a partial fragment of the middle phalanx of the index finger. Thus, the victim was positively identified with absolute medical certainty.

Shortly before trial, the defendant stipulated to the murder and to the identity of the victim; a plea of insanity was entered. A jury found her sane and, therefore, guilty of first-degree murder.

**Murder, Identification, Burned Remains**

# H64 Material Culture Analysis in Forensic Cases: A Call for Formal Recognition by Forensic Anthropologists

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After attending this presentation, attendees will learn about precedents of anthropologists conducting material culture analysis for forensic identification and humanitarian investigations, they will appreciate the particular challenges faced, skills, and knowledge required and importance of both formal training and formalization of expertise in this endeavor.

This presentation will impact the forensic community by publicly addressing an aspect of forensic anthropological practice that has yet to be acknowledged and discussed in a detailed manner amongst practitioners. Continued neglect of the methods and objectives of material culture analysis by forensic anthropologists may lead to false or unwarranted conclusions in court or accusations of malpractice. It will be argued that standardized training and methodology are lacking and should be instituted to meet court requirements not only as they pertain to expertise but also to address concerns highlighted in rulings such as *R* v. *Mohan* and *Daubert* v. *Merrell Dow*.

This presentation advocates enhanced material culture analysis by forensic anthropologists for medico-legal investigations of identity, criminal, and civil investigation. Material culture, particularly that associated with other cultures and countries but also marginalized members of our own society, is currently practiced though poorly documented and rare discussion by anthropologists or other forensic scientists. Exceptions include early experimentation on decomposition by Morse and Daily (1985) and publications of this past year by Baraybar (2008), Birkby et al. (2008) and Daéid et al. (2008). The study of clothing and personal objects compliments other analyses performed by forensic anthropologists towards the determination of time since death, personal identification, and wound type. The examination of these materials can also contribute to a determination or estimation of sex, gender, stature, physique, age, ancestry/ethnicity, and minimum number of individuals. Analysis can validate or call into question physical anthropological methods and results.

Anthropologists have, can, and should conduct material culture analyses to various degrees. The level of action and participation is often dependent upon the context in which anthropologists are employed. At the Centre for Forensic Research at Simon Fraser University in British Columbia, clothing is often passed to the anthropologists along with the remains, following inspection by police, a pathologist, and the coroner. Results of analyses of these associated objects are included in forensic anthropology reports. Similar efforts are discussed—albeit in little detail—by Birkby et al. (2008) to establish a "cultural profile." They use information gleaned to help distinguish unidentified remains of illegal border crossers from Hispanics that are legally within the U.S.

In the investigation of wide-scale human rights violations, Baraybar et al. (2007) and Baraybar (2008) demonstrate the success and limitations of such analysis by anthropologists but also the necessity in environments where resources, including DNA technology, antemortem medical and dental records, may not be available for personal identification efforts. In the experience of these authors, even in wealthy

countries medical or dental records may be non-existent or unavailable due to jurisdictional concerns and protocols or legal obligations of the protection of privacy of personal information. Thus the role of material culture plays a greater role. The same challenges that had been experienced in Kosovo, Peru, and Guatemala were also true in Iraq, despite the exceptionally generous funding from the United States government. In Iraq, however, the employment of a cultural anthropologist to examine material culture appears to have been an appropriate precedent.

In the context of repatriation of soldiers who have died in combat, where criminal prosecutions do not apply, police and pathologists are typically not involved. Drawing conclusions about identity (e.g., nation, military unit or division, rank, etc.) is therefore left to those conducting recovery and analysis of remains based on material culture and osteological analysis (e.g., archaeologists and anthropologists).

In such instances where a significant amount of time has passed or in conditions that promote quick decay of a body, material objects may preserve longer and/or better than osteological remains. The extended preservation of material remains may occur in regions with acidic soils, local conditions that promote dynamic site transformation, and places where perpetrators or civilians seeking the remains of loved ones have disturbed graves. Thus, where the condition of human remains is such that osteological analysis is severely limited, material culture analysis again becomes of primary importance and yet experience has shown that it is sometimes neglected by other professionals of various specializations and appears to be the formal domain of none.

For those with experience in these contexts it stands to reason that they have the requisite experience to conduct similar analyses in the context of domestic forensic investigations. It is argued that the person with the expertise, and thus responsibility for examining clothing and personal objects, is not well established; at times being performed by the police, pathologist, anthropologist or, in some cases, not at all. Anthropologists and archaeologists are generally trained and have experience in the analysis and interpretation of material culture created by various sectors of different societies. Forensic anthropologists are urged to assume a larger, more comprehensive role in the documentation and analysis of material culture.

**Forensic Anthropology, Material Culture, Identification**

## H65 Training the National Disaster Victim Identification Team

*Lucina Hackman, MSc\*, Dundee University, WTB/MSI Complex, Dow Street, Dundee, DD1 4AH, UNITED KINGDOM*

After attending this presentation, attendees will have an understanding of the reasons why the training for the National Disaster Victim Identification Team was established within a United Kingdom university and how this training has helped to move the United Kingdom to the forefront of mass disaster preparation.

This presentation will impact the forensic community by giving those at the forefront of the response to mass disasters another perspective on how training can help to prepare teams for these unexpected and often devastating events.

The Centre for Anatomy and Human Identification at the University of Dundee hosts the advanced course in Disaster Victim Identification for the United Kingdom police. The 500 officers who have completed this course continue on to become members of the National DVI Team. The course offers a postgraduate award in DVI for those who complete the program successfully.

Each mass disaster occurs through a unique set of circumstances and, therefore, requires an individual, tailored response. Every disaster response shares the need to identify those who have lost their lives during, and as a direct result of, the disaster. The identification of the

victims of any disaster is vital for both legal and ethical reasons and helps to provide closure for those families that have been affected. Under the Civil Contingencies Act 2004 the police in the United Kingdom are considered to be one of the primary responders to any disaster involving British nationals and, as such, form the core of any multidisciplinary team whose efforts are aimed at furnishing the identities of those who have died, both nationally and internationally. Other members of the team can include, as required, pathologists, odontologists, and anthropologists. In the past, training for the police has fallen to officers who have gained experience during a response to a previous disaster. This has resulted in a tendency to train as if responding to that most recent disaster, and has also led to a gradual loss of skills through the retirement of those officers. The Centre for Anatomy and Human Identification has moved away from this approach by providing a course which not only aims to produce a cadre of omni-skilled officers, but which also allows police officers to train alongside the specialists, such as the pathologists and anthropologists with whom they will be deployed.

The immediacy of response to these disasters, which is driven by the unexpected nature of the incident, means that police officers must be prepared and trained to the highest standards. This training must be consistent, robust, and fit for purpose. The events of 2005, which included the aftermath of the Asian Tsunami, the London bombings, hurricane Katrina, and the Pakistan earthquake, underlined the need for a recognized group of police officers who would be able to respond to disasters both abroad and on the United Kingdom mainland. In the United Kingdom a cadre of 500 officers has been identified who will form the National DVI team in the event of a deployment. These officers are chosen from all of the police forces in the United Kingdom and proportional representation allows for all of the forces to take an equal share in the provision of officers. The Centre for Anatomy and Human Identification at the University of Dundee provides core training for those officers who have been identified as members of the National United Kingdom DVI Team and who would be required to respond in the event of both national and international disasters. This training also brings together the specialists who would make up the DVI team in the event of a disaster, allowing these two disparate but essential groups of professionals to meet and develop skills together.

**Disaster Victim Identification (DVI), Training, Police**

## H66 The Need for Holistic Investigations of Human Rights Violations: An Example From Peru

*Jose P. Baraybar, MSc\*, EPAF, Av. Mello Franco 341, Jesus Maria, PERU; and Ellen Salter-Pedersen, MA, Department of Anthropology, Indiana University, 701 East Kirkwood, SB130, Bloomington, IN 47405*

The goal of this presentation is to discuss a context based approach to a forensic investigation of human rights violations. The case example from Peru illustrates a comprehensive investigation by a team of forensic anthropologists which was carried out with limited access to resources, facilities, and technology.

This presentation will impact the forensic community by showing an alternate approach to the highly specialized and compartmentalized investigations done in North America and Europe.

This approach has been used in Peru where resources and political will are limited. Exhumations are frequently carried out in remote settings; technology and facilities both in the field and the lab are limited to the most basic equipment. Experts are often trained in the field rather than in academic settings and are required to fulfill multiple investigative roles rather than specialize in one area. These challenges require a different approach than the highly specialized, compartmentalized investigations done in North America and Europe; thus, forensic

anthropologists in Peru have developed a creative approach relevant to the situations in which they work.

A case study is presented based on an investigation carried out in early 2008 by the Equipo Peruano de Antropologia Forense (EPAF). The site is located in a remote part of the Andean highlands of Peru; in 1984 during the internal conflict (1980-2000), over 100 peasants who had been displaced by the terrorists were relocated by the military. They were later killed and buried in a mass grave.

Anthropologists began the investigation with the collection of antemortem data from the friends and relatives of the victims. Antemortem data includes physical descriptions of the victims, the clothes that they were last seen in, any personal effects they possessed, details of their life such as accidents and injuries that may be reflected in the skeletal remains, and details of their disappearance or execution. The information was collected using standardized forms including drawings, images, and color charts. DNA samples were also collected from family members. Interviews with family members were carried out in their native language, Quechua. The data is stored in EPAF's Forensic Anthropology Database which allows for complex searches and protects the identities of the informants.

The goals of the fieldwork were not simply to recover the human remains and evidence; but to reconstruct the most probable scenario. The remains were exhumed using traditional archaeological techniques, commingled bodies were separated in the field before recovery, and skeletal remains were recovered within the clothing when possible. The area surrounding the grave was searched for evidence and to identify possible access routes. Bullets and casings were flagged in the field and were identified as belonging to two types of military issue weapons. The angle of ejection of casings from a similar weapon was tested in both single shot and burst and the results were extrapolated to the evidence found at the site to identify the locations of the shooters and reconstruct the scenario.

Laboratory work included analysis of skeletal remains to develop a biological profile. Trauma analysis was complemented by macroscopic examination of the clothing in order to ascertain the most probable cause of death. Gunshot wounds and sharp force trauma frequently penetrate the clothing; thus, proposed trajectories may be substantiated. Bullets and casing were also found within the clothing. In some cases, pseudotrauma in the bones was confirmed by the lack of evidence in the clothing; conversely, possible injuries were identified in clothing when skeletal remains were incomplete. Detailed descriptions and photos of the clothing were also made for identification purposes.

After laboratory analysis was completed, the antemortem and postmortem profiles were compared to establish putative identifications. These identifications will be confirmed through DNA whenever possible, but frequently immediate family members are not available to provide reference samples. In this case, entire families were killed and DNA may identify relatives among the victims. Exhibitions of victims' clothing and personal effects for recognition by family members may also confirm putative identity.

The holistic approach employed by EPAF has resulted in identifications in multiple cases from the internal conflict in Peru. Once identified, the remains can be repatriated to family members, an important step in the reconciliation process. The recent sentence against the Colina group operatives in the La Cantuta case was a hallmark in Peruvian history as it was the first conviction for human rights violations based on forensic evidence. These achievements indicate that forensic anthropology can succeed in spite of limited resources to funds, specialized expertise, facilities and technology. Further, forensic investigations do not always need to be conducted by highly specialized academics; context based expertise often provides a more comprehensive perspective.

**Human Rights Violations, Forensic Anthropology, Peru**

## H67 Reconciling the Discrepancy in Victim Number Between the S-21 Prison and the Choeung Ek Killing Fields of Cambodia

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After attending this presentation, attendees will learn of on-going investigations relating to the Cambodian conflict of the 1970s and how the minimum number of individuals can be determined in specific mass death scenarios.

This presentation will benefit the forensic community by contributing to the understanding of genocidal conflicts and international human rights investigations.

In 1975, the Communist Party of Kampuchea (CPK) overthrew Lon Nol's Republican regime, ending Cambodia's civil war. The CPK, known as the Khmer Rouge, assumed power led by the notorious Pol Pot. Over the next four years, the CPK inflicted a cultural revolution of forced "Khmerization" on the Cambodian population, which targeted all other ethnicities, nationalities, religions, and languages for eradication. Pol Pot's revolution resulted in the deaths of an estimated 1,100,000 Cambodians. On January 7, 1979, Vietnamese forces reached Phnom Penh, Cambodia's capital, effectively ending Pol Pot's reign.

Negotiations between Cambodia and the United Nations to strike a formal investigative tribunal have finally reached fruition. The first court of its kind in the world, the Extraordinary Chambers in the Courts of Cambodia (ECCC) is a hybrid tribunal that uses local law but incorporates international judges and staff. The ECCC indictment of Kaing Guek Eay (a.k.a "Duch"), the commander of the S-21 prison, has prompted a reexamination of the documentation and forensic evidence associated with his command. One question raised is the apparent discrepancy between the number of victims reportedly processed and killed in S-21 and the number of individuals recovered from the purported burial site, a Chinese graveyard near Choeung Ek. At least 12,499 individuals were reportedly killed following their incarceration at S-21, while the remains of only 8,895 victims have been recovered to date at Choeung Ek. This discrepancy raises multiple possibilities, including the potential of alternative burial sites or a greater number of survivors than previously believed.

References to S-21 first appear in September 1975 in Khmer Rouge documents but the facility did not become operational until May or June of 1976. Kaing Guek Eav arrived at S-21 in June 1976 and retained control of the facility until the arrival of the Vietnamese in January 1979. Duch also served as the Security Chief of the CPK's state security organization known as the *Santebal*. No specific documents directly linking Pol Pot to orders to torture or execute individuals at S-21 have been found, although links between S-21 and the Party Center (*Angkar*) have been established conclusively.

All prisoners at S-21 were killed not only because of their perceived crimes but also to maintain secrecy regarding the prison's existence. The number of prisoners executed at Choeung Ek fluctuated daily from a few dozen to over three hundred. A review of the confessions and other documents recovered from S-21 placed the total number of prisoners at 12,499. Of the thousands of prisoners processed at S-21, only seven are known to have survived. Given the uncontested link between S-21 and Choeung Ek, why are more than 3,500 victims still unaccounted for? A review of the available evidence reveals the following:

1. When S-21 first opened in 1976, prisoners were clubbed to death in the field to the west of the compound and buried where they fell in shallow graves. This impromptu cemetery quickly filled and by 1977, Choeung Ek became the killing field and burial facility for S-21. Remains from the S-21 compound have been recovered and in 2008, additional remains were found during construction at the property adjacent to the prison. A formal estimate of the minimum

number of individuals (MNI) represented by these remains has not yet been calculated.

2. Mass graves at Choeung Ek were exhumed beginning in January 1979 under the auspices of the government's Genocide Research Committee into Khmer Rouge crimes. Local communities also exhumed mass graves, and a small number of graves were robbed in search of valuables. Of the 130 purported mass graves at Choeung Ek, only 89 have been formally excavated. An up-to-date survey of Choeung Ek using more advanced imaging techniques is warranted.

3. Exhumations at Choeung Ek have uncovered a total of 8,895 individuals. However, this MNI was calculated by counting complete skulls. A more exacting method of determining MNI is needed to accurately estimate the victims represented.

Despite the initial perception of a significant number of missing victims, it is highly probable that the overwhelming majority of reported victims from the S-21 prison can be accounted for, provided additional forensic investigations are carried out at both the S-21 prison and the Choeung Ek killing fields. In spite of the more than 30 years that have past since these events took place, scientific examination of physical evidence can play a vital role in answering crucial questions in the upcoming legal proceedings.

**Forensic Anthropology, Human Rights Investigations, Genocide**

# H68    Forensic Findings on Illegal Burials in Colombia

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The goals of this presentation are to: (1) present significant forensic findings on illegal burials secondary to the armed conflict in Colombia, (2) describe the types of illegal burials specific to different illegal groups involved in the armed conflict in Colombia, and (3) to analyze illegal burials in a sociocultural context.

This presentation will impact the forensic community by explaining the analysis performed on illegal burials by forensic experts under the context of the armed conflict in Colombia, and the sociocultural characteristics of the population that allowed the detection of forensic evidence on the victims of illegal groups.

The armed conflict in Colombia started over 50 years ago involving several illegal groups, including left-wing guerrillas (Revolutionary Armed Forces of Colombia, FARC; National Liberation Army, ELN) and right-wing paramilitaries (United Self-Defense Forces of Colombia, AUC). Over 30,000 people have disappeared over the last two decades secondary to the action of these illegal groups. For that reason, forensic experts in Colombia are challenged with unusual findings in illegal burials, including countless types of death, funerary rituals, and entombments. This presentation has the objective to show and describe some of the more important findings revealing several sociocultural traditions on illegal burials, including religious beliefs and magical thoughts. In addition, illegal burials have revealed that specific groups adopt different criminal methods and techniques (apparently during their basic induction to the armed group) including anatomic disarticulation of the bodies recovered in different geographical places from Colombia.

Several findings on illegal burials are important for the forensic experts in Colombia: When a member of the guerrilla group dies, the funerary ritual depends on their rank inside the organization, taking into account their religious and magical beliefs, and commonly emphasizing eternal life and revenge.

There are reports of illegal groups collecting anatomic parts (usually bones) of kidnapped people who died in captivity. Their objective is to collect money from the victim's family in exchange for information of the exact place of inhumation. These anatomic elements

and the information given by the family are very key elements in the preliminary identification of exhumed remains.

Human bodies belonging to important members of the illegal group are often covered using five layers of different materials to ensure a longer preservation period. This practice has also been documented in cases of illegal burials of important persons in the society. Additionally, materials like bullets, ropes, handcuffs, knifes, clothes, liquor bottles, syringes, gauzes, and bandages are frequently found in the site of burial. These elements are very important to determine the circumstances of death.

Analyses of illegal burials by forensic experts are critical in determining the illegal groups involved in the crime and the identification of the disappeared persons. Proper knowledge of the groups participating in the armed conflict, as well as the diverse sociocultural patterns of the population living in Colombia, are important elements in the forensic field.

Colombian forensic experts are working hard to return the remains of missing persons to their families and are collecting evidence for criminal justice. Forensic sciences are essential to investigating the truth about the violent crimes in Colombia.

**Colombia, Illegal Burials, Armed Conflict**

# H69    Evolution of Forensic Archaeology and Anthropology in Italy: Three Criminal Cases

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After attending this presentation, attendees will be briefed on the first three cases where forensic archeology was applied in Italy for the search and recovery of victims of organized crime, and how the scenario is evolving.

This presentation will impact the forensic community by showing the application and advantages of archeological methods to the search and recovery of buried corpses.

Forensic archeology applies the techniques of search and recovery commonly performed in the archeological context to forensic cases, and aims at the best possible conservation of the deposition site and the human remains. Thus it should be considered of primary importance by police and judicial authorities in cases where a body needs to be found and adequately recovered. Nevertheless, in Italy and in southern Europe this discipline is still rarely applied in real cases, with obvious disastrous outcomes.

The first case occurred in October 2001 in a wooded area at the outskirts of Milan. Children from a nearby nursery school found a boot emerging from the ground that appeared to contain several bones. The skeletal remains were recovered as indicated by archeological recommendations; a conservative recovery of all the skeletal elements, clothes, and personal belongings was then performed. The area was recorded by topographical methods, and tree roots crossing the deposition site were sampled, which made a postmortem interval estimation (PMI) possible. The botanical and product analysis of clothes limited the PMI estimation to between 1995 and 1998. The reconstruction of biological data revealed that the skeletal remains belonged to a 20-25 year old female subject. A facial reconstruction was performed and the resulting broadcast helped a prostitute from Kosovo recognize the subject as a friend called Viola, but at the present time a

positive identification has not yet been achieved. The anthropological analysis pointed out a cut mark with peri-mortem characteristics at the lower margin of the 10th left rib.

The second case concerns the corpses of two missing adolescents found in May 2004 in a wooded area near Varese. The discovery came after a confession from their murderers, called "Satan's Beasts" because of their devotion to drugs and Satanism, who indicated the woods in which the victims had been buried. The first recovery procedures were conducted by cadaver dogs, followed by field walking, and the use of a metal detector/georadar. After no signs of burial were found, the murderers were led to the site and indicated a new field, where the application of archeological methodology led to the appearance of a ditch. The fill was then removed and on the bottom two skeletonized corpses were found. The first subject was male, 15-18 years old, and the second one was female, 17-22 years old. The two corpses were identified as the missing adolescents according to odontological and anthropological methods. The female subject was hit at least 11 times by a sharp force tool (a large knife). The male subject was hit at least 12 times and was also hit by a mallet in the facial region. The lesions observed were consistent with the events referred to by the murderers.

The third case concerns the search of a buried body (a victim of a mafia execution) which, according to the murderer, took place in December 2006 in a wooded area in the outskirts of Milano. His accomplices reported he had shot and beheaded the victim, although he had always denied it (he in fact had said that he was only responsible for burying the body, not for the murder). Thus, the magistrate wanted to verify whose version was true. At the beginning of the search, a 10 x 10 m area was defined the analysis of soil anomalies went on until excavation activities revealed the cranial vault. The human remains in the fill were cleaned, photographed, sketched, and then a 3-D digitizing analysis of the site was performed. Close to the cranium, a dental prosthesis was found. The subject was male, 20-28 years old, according to anthropological analyses. Positive identification was reached by odontological data based on the prosthesis found during the excavation procedure. The cranium, severely fractured, was finally cleaned and reassembled in order to verify the presence of lesions. The analysis of the cranium pointed out that the victim was hit by two gunshots. No lesion consistent with a possible beheading was observed.

These cases show how, at least in northern Italy, judges and police authorities are beginning to employ anthropologists and archeologists for the retrieval and recovery of buried bodies. Among pathologists and magistrates, these cases have also strengthened the theory that only by proper archaeological methodology can proper reconstruction of identity, mode of deposition and trauma analysis be performed.

**Forensic Anthropology, Forensic Archeology, Buried Corpses**

## H70     Sexual Dimorphism of Index to Ring Finger Ratio in South Indian Children

*Tanuj Kanchan, MD\*, Kasturba Medical College, Department of Forensic Medicine, Light House Hill Road, Mangalore, 575 001, INDIA*

After attending this presentation, attendees will be able to recognize index and ring finger ratio as a possible sex indicator in South Indian children.

This presentation will impact the forensic community in the identification of victims from dismembered human remains encountered in cases of mass disasters and explosions, and assault cases where the body is dismembered to conceal the identity of the victim. When an individual hand is recovered and brought for examination, index and ring finger ratio may prove to be a useful tool for sex determination of the dismembered remains.

Definitive sexual traits are not manifested until after the full development of secondary sexual characters that appear during puberty.

Thus sex determination from prepubertal human remains is a challenge for forensic experts and physical anthropologists worldwide. The research was undertaken to investigate sexual dimorphism of the index and ring finger ratio in South Indian children.

The study was conducted on 350 children (175 males and 175 females) of South Indian origin aged 12 years and below at Manipal in coastal Karnataka, South India. The index finger length (IFL) and the ring finger length (RFL) were measured in millimeters in each hand. The distance between the mid point of the proximal most flexion crease at the base, and the most forward placed point (tip) of each finger in the midline on the ventral (palmar) surface were recorded for each hand. The index and ring finger ratio was computed by dividing index finger length by ring finger length. The data obtained were analyzed statistically using SPSS (Statistical Package for Social Sciences, version 10.0) computer software. Student's t-test was performed to compare the index and the ring finger lengths and the ratio in the two hands, and between both sexes; a p-value $\leq 0.05$ was considered as significant.

Mean IFL and RFL values were significantly higher in males for both hands. Difference in mean RFL between males and females was, however, more than the difference in mean IFL in both hands. In all the hands that were examined, mean RFL was greater than mean IFL in both males and females. Index and ring finger ratio derived from the finger lengths ranged from 0.89 to 1.02 in males with a mean of 0.9500, and from 0.90 to 1.06 with a mean of 0.9887 in females for the right hand. For the left hand, the index and ring finger ratio ranged from 0.89 to 1.02 in males with a mean of 0.9488, and from 0.90 to 1.06 with a mean of 0.9864 in females. The derived ratio showed a statistically significant difference between males and females ($p \leq 0.001$). The index and ring finger ratio did not show any significant differences between the two hands in males and females.

The study reveals that the index and ring finger ratio shows sexual dimorphism in the South Indian children and thus may prove useful to determine the sex of an isolated hand when it is submitted for medicolegal examination. The index and ring finger ratio is found to be higher in females when compared to their male counterparts in both hands. The study suggests that ratio of 0.9700 and less is suggestive of male sex for both hands, while a ratio of more than 0.9700 is suggestive that the hand is of female origin. Similar studies are proposed to confirm the findings of our study and find if sexual dimorphism in the index to ring finger ratio is a constant feature in other population and age groups.

**Forensic Anthropology, Sex Determination, Index to Ring Finger Ratio**

## H71     Subadult Sexual Dimorphism in Long Bone Dimensions (The Luis Lopes Collection)

*Miriam E. Soto, MA\*, The University of Tennessee, 250 South Stadium Hall, 1425 South Stadium Drive, Knoxville, Tennessee 37996*

After attending this presentation, attendees will learn how significant sexual dimorphism is present in subadult long bone dimensions. Attendees will also learn that the development of a new sex determination method for subadults, utilizing long bone dimensions, may be a possibility in the future.

This presentation will impact the forensic community by contributing to the available research on subadult sex estimation. This research is important because sex estimation is an important aspect of the biological profile. However, when dealing with subadult remains sex assessment is still very difficult. Development of a reliable sex determination method for subadults would aid in the identification of unidentified juvenile remains.

The purpose of this study is to evaluate sexual dimorphism of long bone dimensions in a sample of 72 individuals ranging in age from birth to 17 years. The sample was obtained from the Luís Lopes collection

curated at the Bocage museum in Lisbon, Portugal. The subadult sample consisted of two groups, one group ranging in age from birth to 11 years and the second group ranging in age from 12 to 17 years. In order to test whether significant sexual dimorphism was present in the long bones, MANCOVA tests were performed on the following measurements: maximum clavicle length, maximum diameter of the clavicle, minimum diameter of the clavicle, circumference of the clavicle, maximum glenoid cavity breadth, maximum diameter of the humerus, minimum diameter of the humerus, circumference of the humerus, maximum breadth of the distal humerus, maximum diameter of the radius, minimum diameter of the radius, circumference of the radius, maximum diameter of the ulna, minimum diameter of the ulna, circumference of the ulna, maximum diameter of the femur, minimum diameter of the femur, circumference of the femur, maximum breadth of the distal femur, maximum diameter of tibia, minimum diameter of the tibia, circumference of the tibia, maximum breadth of the proximal tibia, maximum diameter of the fibula, minimum diameter of the fibula, and circumference of the fibula. Results of the analysis indicate that significant sexual dimorphism was detected in the clavicle, radius, and tibia of the young subadult group. In the older subadult group, significant sexual dimorphism was detected in the clavicle, humerus, ulna, femur, and tibia. Logistic regression tests were also performed on the data to determine the probability of classifying an individual into the correct sex group. Cross-validation was not available due to small sample sizes. Therefore, all probabilities are inflated and results should be considered preliminary. Although the probabilities are inflated, the results suggest that the sexual dimorphism in subadults may be significant enough to differentiate between the sexes. Clavicle measurements proved to be fairly accurate sex predictors for young and older subadults. Models constructed from clavicle measurements correctly sexed ~83% of the young subadult sample ($\leq$11 years) and ~95% of the older subadult sample ($\geq$12 years). Humerus, tibia, and ulna measurements were more accurate at predicting the sex of older subadults than younger subadults. Humerus measurements correctly classified ~77% of the younger subadult sample and ~94% the older subadult sample. Ulna measurements correctly classified 74% of the younger subadults and 86% of the older subadult sample. Although only ~72% of the young subadult sample was correctly sexed using tibia measurements, they appear to be very effective (~92% accurate) for predicting the sex of older subadults. Overall, the most accurate models for sexing subadults in this sample were constructed from the measurements of several long bones, but this greatly reduced the sample size. Approximately 91% of the young subadult sample was accurately classified using a combination of measurements from different long bones.

This research suggests that sexing subadults from long bone dimensions could be a possibility in the future. Further research should be conducted on sexual dimorphism in subadult long bone dimensions. Such research could lead to the development of a sex determination method for subadults.

**Subadult, Sexual Dimorphism, Long Bone Dimensions**

## H72    Sex Estimation From the Clavicle in Modern Americans:  Traditional Versus Alternative Approaches

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The goal of this presentation is to illustrate several approaches to quantifying sexual dimorphism in the human clavicle: linear discriminant functions, neural networks, and curvature analysis with medial axis representation.

This presentation will impact the forensic community by providing discriminant functions for sex estimation from the clavicle in modern Americans. Additionally, this research aims to combine information about size and shape to achieve maximum accuracy in sex estimation and to provide a model for similar analyses of other skeletal elements.

Traditionally, linear discriminant functions are used to discriminate among the sexes. However, neural networks offer the power of multiple non-linear discriminant functions and often provide superior discriminatory capabilities. Furthermore, capturing shape offers an additional method of discriminating sex that, when combined with either of the methods above, can assist in making accurate assessments. This presentation will examine each of these methods and propose the most accurate and precise method for sex estimation.

The William F. McCormick Clavicle Collection at the University of Tennessee contains clavicles from approximately 2,000 individuals from East Tennessee. In order to facilitate rapid data collection from a sample of this size, the entire collection was scanned with computed tomography scanning, and three-dimensional computer models were created. Scanning the bones also enabled the exploration of a number of alternative measurements and way to standardize the curvature analysis. A subset of 1,407 adults was used in this study. The sample was comprised of 53 African Americans and 1,354 Caucasians. Measurements analyzed include maximum length, sagittal midshaft diameter, vertical midshaft diameter, maximum midshaft diameter, minimum midshaft diameter, two diameters from the lateral end, and two diameters from the medial end. Curvature was analyzed by making angular measurements on a medial axis representation of the clavicle shaft.

The sample was analyzed with pooled and separate ancestries, and all discriminant analyses were performed in SAS 9.1 using cross-validation. Overall, the hit ratios were the same with both approaches, but separate discriminant functions are provided for whites and blacks. The best classification rate was 92.22% using maximum length, maximum and minimum midshaft diameters, and maximum and minimum diameters of the lateral end. A simple three-variable model with maximum length and maximum and minimum midshaft diameters discriminated with 91.44% accuracy. The new measurements from the lateral clavicle discriminate 77.72% accurately alone. The medial measurements did not perform well (62.38%) and were omitted from all final models. Maximum and minimum midshaft diameters performed equally as well as sagittal and vertical midshaft diameters; since maximum and minimum midshaft diameters are easier to measure precisely, these measurements offer a way to reduce inter-observer error.

The same sample was subsequently classified using a Feed-Forward Backpropagating Neural Network (NN). A network was constructed using all nine measured variables plus one binary variable indicating side (L = 0, R = 1) as input nodes, followed by a layer of eight hidden nodes, and finally an output layer of two nodes (one per sex). The network was trained using random subsets of the clavicle sample, where 30% were used for training, 30% were used for validation, and 40% were used for

testing. Neural networks are ideal for a sample of this size because they are robust to assumptions of normality and equality of variance-covariance matrices. The final testing classification rate was 94.58%, while the overall classification (including the whole sample) was 94.22%. The three-variable model with maximum length and maximum and minimum midshaft diameters resulted in a testing classification rate of 93.48%.

Analysis of curvature was performed using a medial axis representation of the clavicle. The bone models were divided into 8-16 sections perpendicular to the shaft. The midpoint of the section was selected as one vertex of the medial axis. Subsequently, line segments connecting the medial axis points were connected to form an approximation to the shaft curvature. By analyzing the angular deviations between adjacent sections, quantitative shape information is acquired in a compact format. Distinct shape trends between the sexes were noted, but require further analysis before they can be suitably integrated into the classification procedures.

**Sex Estimation, Clavicle, Neural Networks**

## H73 The Impact of Racial Metric Variation in the Pelvis on the Morphological Assessment of Sex

*Ginesse A. Listi, PhD\*, Louisiana State University, 1723 Lombard Drive, Baton Rouge, LA 70810*

The goal of this presentation is to explore whether or not racial metric variation in the pelvis affects the morphological assessment of sex.

This presentation will impact the forensic community by demonstrating that quantitative variation in the pelves of white and black Americans does not affect the accuracy of sex determination.

Previous research on determining race from the pelvis generally has focused on quantitative variation. Based on certain measurements of the pelvis, or of the os coxa, researchers have been able to discriminate race between white and black Americans in test samples with overall accuracies ranging from 58% to 83%. Also, several of these studies have found that the pelves of black Americans tended to be "smaller" or "narrower" than those of white Americans, though such differences were not always statistically significant.

Determination of sex from the morphology of the pelvis is based on the premise that, in many of the traits, females are larger (longer, wider, broader) than males. Yet, few studies have assessed whether the quantitative differences between the races affect, or are apparent in, the morphological traits used by anthropologists to determine the sex of the individual. In one notable exception, Patriquin et al. (2003) found significant differences between white and black South Africans in the accuracy of morphological traits for sex determination.

The purpose of the present study is to examine whether or not sex determination based on morphological traits is impacted by racial quantitative variation. Nineteen morphological traits were evaluated by two different observers in 876 os coxae. For this study, a random sample of 400 individuals was selected, composed of 100 of each race and sex category. Chi-square was used to assess the impact of race on: (1) the accuracy of sex determination based on all 19 traits collectively, (2) the evaluation of sex for the individual traits, and (3) inter-observer variation in sex assessment. The level of significance for all tests was $p < .05$.

Results indicate that race did not impact the accuracy of sex determination for either observer when all traits were considered together. However, race did affect the evaluation of sex for some individual traits. For Observer "A," significant variation existed in sex assessment between black and white individuals in 6/19 traits for males and 1/19 traits for females. For Observer "B," no significant difference in sex assessment between black and white males was noted for any trait;

however, significant differences in sex determination existed in 3/19 traits in females. In the analysis of inter-observer variation, significant inter-observer differences were noted between the races for females when all traits were considered together, but not for males. When traits were assessed individually, 2/19 traits in males and 4/19 traits in females had significant inter-observer differences in sex determination between the races.

Though race affected the evaluation of sex in certain individual traits, the accuracy of sex determination was not impacted when all traits were considered collectively. Additionally, the traits that showed significant variation in sex assessment between the races generally were not related to the size of the pelvis, or to the pelvic inlet or outlet, as might be expected considering the reported size differences between white and black pelves. Instead, the traits affected by race were "discrete" (i.e., they are commonly evaluated as "present/absent"). Furthermore, of the nine traits that showed significant differences in sex assessment between the races, none overlapped between the observers; however, five of these traits were significant in the analysis of inter-observer variation. These results may indicate that the disparity in sex determination found in certain traits may be due as much to inter-observer differences in trait interpretation as to morphological dissimilarities between the races.

In conclusion, the objective of this research was not to be able to determine race from the pelvis, but to explore whether or not the quantitative differences in the pelves of white and black Americans affect the morphological assessment of sex. Results from this study indicate that they do not.

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**Race, Sex Assessment, Pelvis**

## H74 Bilateral Asymmetry in Historic Versus Modern Skeletal Remains: Activity and Identification

*Shannon E. May, MA\*, 250 South Stadium Hall, Department of Anthropology, University of Tennessee, Knoxville, TN 37966*

The goal of this presentation is to determine the presence of bilateral asymmetry in two temporally and geographically diverse skeletal collections, and to compare and contrast these values. Levels of lateralization are then considered in terms of activity, sex, and age.

This presentation will impact the forensic community by demonstrating the utility of bilateral asymmetry in modern forensic casework and in skeletal biological contexts. Furthermore, the population-specific nature and sexual dimorphism of side-dominance is addressed.

Bilateral asymmetry is defined as metric and morphological differences in paired biological structures, and may be used to infer various forms of environmental and mechanical stress imposed upon the skeletal system.[1] Directional asymmetry, also termed lateralization or side-dominance, is observed most often in human skeletal remains, and occurs when unimanual loading induces bone modification on a particular side. This typically results in larger size, cortical thickness, and greater robusticity of bones from the side receiving the greater stress. Anthropological studies have used bilateral asymmetry to establish activity patterns,[2] differentiate between the sexes,[3] and assign handedness to an unknown set of skeletal remains.[4]

The current study investigates the level of asymmetry in two diverse skeletal collections: (1) The New Lisbon Collection sourced from Lisbon, Portugal, representing a historic European sample, and (2) The William M. Bass Skeletal Collection, sourced primarily from the

southeastern United States, representing a modern skeletal set born during the twentieth-century. Equal sample sizes of 250 individuals from each collection were used. Postcranial long bone measurements were taken on each sample according to standards created by Moore-Jansen et al.[5] A total of seventeen measurements were taken, evaluating maximum and minimum length, diameter, and areas of muscle attachment. Both right and left elements were measured, using an osteometric board and digital calipers. An asymmetry index was also calculated as the difference between right and left elements of a pair, standardized by the mean of both components: $[(R - L) \times 100] / [(R + L)/2]$. This formula preserves both direction and magnitude of asymmetry, while standardizing for size, thus allowing comparison between skeletal areas and individuals. Extensive biographic information has been documented for each skeletal sample, allowing additional demographic analysis.

Paired T-tests revealed significant side-difference ($p \leq 0.05$) in twelve measurements in the New Lisbon Collection but only five variables in the Bass Collection. While the Lisbon samples demonstrated significant side discrepancy in variable dimensions, Bass samples were primarily bilateral in terms of maximum length. The New Lisbon Collection also showed a greater sex-specific concentration in asymmetry: female asymmetry located in the mid-arm (measurements of the elbow-joint) and male asymmetry concentrated in the shoulder girdle. Sexes were separated for between-collection comparisons, and only shared asymmetrical variables were utilized. Multivariate Hotellings 2-sample T-tests showed Lisbon males having significantly higher levels of asymmetry in all variables except ulna length ($p = 0.002 - 0.01$), as did Lisbon females ($p = 0.001 - 0.04$). In two measurements, the average Bass male asymmetry fell within the range of the Lisbon females, underscoring the importance of source/context when investigating sex and asymmetry in unknown remains. Additional analysis on the New Lisbon Collection reveals that Portuguese juvenile remains demonstrate lateralization by ten years age-at-death, with subadult males having higher asymmetrical values.

It may be concluded that bilateral asymmetry is population-specific and most likely related to the amount and degree of mechanical loading experienced by the group. Modern groups like the Bass Collection represent a more sedentary lifestyle, resulting in less activity-induced modification. Conversely the historic Portuguese represent a group habitually engaged in heavy labor. While sexual dimorphism in asymmetry exists for each group, it is crucial to discern the potential sample populations and the inherent levels of asymmetry before assigning sex or handedness to an unknown set of remains. Finally, analysis of juvenile remains indicates that lateralization in long bones may be apparent at a young age, particularly in highly active groups. This may provide another approach to the difficult task of determining sex in subadult remains.

**References:**
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2. Fresia AE, Ruff CB, Larsen CS. Temporal decline in bilateral asymmetry of the upper limb on the Georgia coast. Anthropological Papers of the American Museum of Natural History 1990;68:121-135
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**Asymmetry, Handedness, Mechanical Loading**

# H75    Observer Error Analysis Trends in Forensic Anthropology

*Megan Ingvoldstad, MA, Ohio State University, Department of Anthropology, 244 Lord Hall, 124 West 17th Avenue Columbus, OH 43210; and Christian Crowder, PhD*, Medical Examiner's Office, 520 First Avenue, New York, NY 10016*

After attending this presentation, attendees will recognize research trends in the evaluation and reporting of observer error in the forensic anthropological literature.

This presentation will impact the forensic community by demonstrating the lack of observer error analysis in the anthropological literature over a 28-year period. Furthermore, this research indicates the trends in types of statistical analyses used for observer error analysis, when they are performed. As issues of professional standards continue to be addressed in the courts, it is imperative that anthropologists be proactive by increasing the scientific rigor in developing, comparing, and testing anthropological methods.

Forensic anthropology has recently emerged from a transitional period in which it has been described as the application of physical anthropological analyses in the medico-legal setting. Methods developed within physical anthropology typically explore research questions at the population level, while forensic anthropology focuses more on the individual of a given population. Furthermore, methods of analysis used in the forensic context must meet specific demands due to the evidentiary nature of the results. While methods developed within physical anthropology have little need to recognize the potential legal and social ramifications of unreliable or inaccurate results, the scientific method (regardless of field) demands a certain level of scientific rigor in method development. Since the *Daubert* court ruling, many forensic disciplines have begun to critically evaluate the methods used in their examinations. The ruling has likely contributed to the increased awareness and interest in quantifying, critically assessing, and re-evaluating some of the techniques most often used by forensic anthropologists. The issue of error rates; however, have been less often addressed. This presentation has two goals: (1) evaluate the trends in the types of statistical analyses used for observer error analysis, and (2) demonstrate the overall deficiency in observer error analysis within the literature to encourage discourse among anthropologists in establishing guidelines in this type of analysis.

Anthropological articles from 1980 through 2008 in the Journal of Forensic Sciences were evaluated. Keyword searches (e.g., method, technique, statistics, error, bias) were utilized initially, followed by manual reviews of all anthropological articles to determine if an analysis of observer error was warranted for the studies. Any paper that introduced, compared, validated, or modified anthropological methods and techniques was considered as potentially needing observer error analyses. Overall, 269 articles fit the criteria for inclusion in this survey. Each article was evaluated and the inclusion or exclusion of intra- and inter-observer error was recorded. In the event that an observer error was evaluated, the statistical technique was recorded.

Out of 269 articles, 189 (70%) provided no analysis of observer error leaving only 80 (30%) that have performed or presented observer error analyses. Furthermore, only 25 studies (9%) performed both intra- and inter-observer analyses. Twenty-nine studies (11%) only documented results from inter-observer analyses, while 26 studies (10%) only provided intra-observer analysis results. Papers reviewed prior to 1986 did not include any analyses of observer error and papers prior to 1990 did not include any analysis of inter-observer error. While it was hypothesized that the amount of studies performing analyses of observer error would increase over time relative to the amount of total research papers published per year, it was discovered that the frequencies simply rise and fall over the years. The types of statistics used over the years to evaluate observer error prove to be just as variable and do not demonstrate any selection trend over time.

As the courts continue to raise the bar regarding professional standards, forensic anthropology must be committed to providing analyses that are of the highest quality. Researchers should carefully select statistical techniques for evaluating observer error and strive to not only identify the error, but explore the cause. Researchers need to be versed in the concepts of bias, repeatability, precision and accuracy so as not to conflate the terms and use them interchangeably. Analyses of observer error are a basic component of the scientific method and assist in evaluating the reliability of our methods. Human variation is a frequent complication in the development of methods within biological anthropology. Differentiating method error from biological variability will assist in providing a baseline interpretation of methods and their variables as useful predictors in skeletal analyses.

***Daubert*, Observer Error, Statistics**

# H76 Prediction of Shoe Size From Tarsals and Metatarsals

*Paul D. Emanovsky, MS\*, JPAC-CIL, 310 Worchester Avenue, Hickam AFB, HI 96853*

The goals of the presentation are to explore the relationship between various elements of the foot and overall foot length, to create regression equations for prediction of foot length in unknown cases, and to assess the correlation of predicted foot length with reported shoe size.

This presentation will impact the forensic community by showing how estimation of shoe size can potentially be a valuable addition to the standard biological profile. Notably, it can help limit the number of antemortem searches required to narrow a pool of potential individuals. It will also assist with issues such as commingling as well as re-associate material evidence from recovery scenes to specific individuals.

Regression formulae have been derived using a sample of white and black males from the Hamann-Todd Collection (HTH) housed at the Cleveland Museum of Natural History. These formulae were tested for efficacy on a subsample of individuals identified at the JPAC-CIL for whom the proper antemortem records and postmortem measurements were available. Currently, footwear vendors provide tables that correlate maximum foot length to shoe size. Although there is some variability in the size of ones' shoe due to manufacturing and stylistic differences, investigators can confer with specific manufacturers to account for such differences in size.

Bony foot elements were measured on a sample of 123 white and black males from the Hamann-Todd Collection for whom antemortem foot length data was available. Twenty-four measurements of various foot elements were collected on the HTH sample for ongoing research projects involving sexual dimorphism and ecogeographical patterning. Logistic regression (LR) was used to create population specific (white or black) and generic (combined white and black) regression models for the prediction of overall foot length. These formulae were then evaluated to determine which single element or combination of elements provided the best predictions of foot length.

For the current study, variable selection from the 24 available measurements followed a heuristic protocol. Elements of the foot are commonly well preserved in archeological and forensic contexts. Among the best preserved elements of the foot typically encountered in JPAC-CIL cases are the calcaneus, first metatarsal, and the cuboid. For example, each of these elements are present in at least 15 individuals out of a 20 individual subsample of identified CIL accessions. This subsample includes foot size data (via shoe size recorded in the individual's medical records) and postmortem bony measurement data. This sample involves individuals from a variety of distinct time periods and, as such, it was used to evaluate the regression equations and assess its broad utility. The potential variable list was further shortened by the requirement of having a high correlation coefficient with foot length.

While all of the metatarsals were found to have higher correlations than tarsals, it is desirable to include a model that incorporates hind, midfoot, and forefoot components. These three components then can be summed, entered using stepwise LR, or used independently in the absence of better correlated elements. The maximum lengths of the calcaneus, cuboid, and first metatarsal were all found to be well correlated with overall foot length. For instance, in the white and black male combined sample the two-tailed Pearson correlations are 0.639, 0.563, and 0.773 respectively. All are statistically significant correlations at the 0.01 level. Using the sum of the cuboid and first metatarsal lengths (sumcubfstmax) the following equation is derived:

Predicted foot length = -6.046 + (sumcubfstmax) 1.333 ($r^2$ = .63, Inaccuracy = 7.16 mm, Bias = -8.85 mm, standard error of model = 9.964). Standard errors of the models as well as correlations are typically improved with population specific equations.

Regression methods for estimating foot length and subsequently shoe size data have been found to be statistically viable. Standard errors of the equations are on par with estimates of stature from tarsals which has proven to be highly useful in variety of forensic and archeological contexts. It is anticipated that prediction of shoe size can become a useful forensic tool within the investigators toolkit if proper antemortem records are available.

**Logistic Regression, Biological Profile, Pedal Analysis**

# H77 Radiography as a Tool for Contemporary Anthropological Research

*Melissa A. Pope, BA\*, University of South Florida, Anthropology Department, 4202 East Fowler Avenue, Tampa, Florida 33613; and Liotta N. Dowdy, BA\*, University of South Florida, 3115 Palmira Street, Tampa, Florida 33629*

After attending this presentation, attendees will gain knowledge of: the role of radiography in forensic anthropology, the potential contribution of radiography as a tool for deriving quantitative measurements in the research setting, problems associated with this application, and potential areas of death investigation that may be improved through research using radiography for quantitative analysis.

This presentation will impact the forensic community by enabling better standards for human identification and by showing that anthropological methods are founded in science, there is a need to validate all methods.

This study assessed whether radiographs can be used to generate accurate and reliable morphometric data, or whether they introduce too much error. This presentation is an effort to uncover research that relates to this topic, and to determine whether radiographs are a valid tool (accurate and reliable) for anthropometric analyses of contemporary populations.

This research will help substantiate the use of radiography to facilitate the generation of population-specific standards for the biological profile that may be used to facilitate forensic death investigations and investigations into war crimes. Radiographs have been used extensively as a means for generating osteological data because of their many advantages, including cost effectiveness and simplicity. In anthropology, radiography has recently and less conventionally been used as a tool to generate samples of contemporary people for developing new quantitative standards. Advantages of this application include: non-invasiveness, the potential for longitudinal research, and the accessibility of skeletal data in fleshed remains. During death investigations, the methods of the investigators invariably fall

under scrutiny, and must be shown to be accurate, reliable and appropriate for what is being analyzed. Therefore, it is necessary that the standards used to build the biological profile are based on samples that are representative of the populations being analyzed. This research will ultimately aid in the development of new and more reliable methods that will contribute to making positive identifications and will withstand scrutiny during trial.

This study derived linear measurements (maximum length, midshaft width, and diameter of the head) from ten femurs, ten humeri, nine ulnae and nine radii (total n=38). The sample was generated from dry bones belonging to USF's anthropology department. The bone measurements were then compared to their corresponding radiographs. The bones were filmed in antero-posterior view, except for the ulnae, which were filmed in medio-lateral view. Specific measurements were selected based on landmark visibility on the radiograph, their ability to be combined into one sample, and their utility in anthropological investigation. Measurements were taken blindly by both authors so that inter-observer error could be identified. Magnification error was accounted for, and problems associated with accounting for the three-dimensional shape of the bone were identified. The samples were combined and Mann-Whitney tests were performed to determine if there were significant differences between the dry bone and the radiograph measurements. These measurements were plotted against each other to see whether or not there was a clear relationship. The distances were then correlated using a Spearman's correlation test. Finally, the differences between the radiograph and the dry bone measurements were computed for all distances and descriptive statistics were calculated for overall assessment of accuracy and precision.

The Mann-Whitney found all three distances to be highly insignificant (p=0.499, 0.884, 1), indicating that the dry bone and radiograph measurements were similar. The plots indicated a fairly clear relationship between measurements, although less so for the width of the head. Spearman's correlations showed all measurements to be significantly correlated (p=0.01; r=0.999, 0.974, 0.98), indicating that the radiograph measurements were accurate. Descriptive statistics of the differences showed that on average the measurements were fairly accurate, but not within the ±2mm range that is preferable for anthropometric analyses.

Despite the lack of accuracy, the results are promising. It is anticipated that the results may improve as this study progresses. This study will be furthered by: quantifying inter- and intra-observer error, analyzing each bone separately, and analyzing whether certain portions of the bone or types of measurements yield more accurate results (as these are affected by difficulties in accounting for magnification error). More accurate ways to account for magnification error and distortion are needed for this methodology. An elaboration of this problem will be provided in the presentation.

**Radiography, Metric Analysis, Osteometry**

# H78    Can Bilateral Joint Asymmetry Be Used as an Estimation of Handedness?

*Kathryn R.D. Driscoll, MA\*, University of Tennessee, 250 South Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will understand whether bilateral asymmetry of the humeral, femoral and tibial joints should be used to estimate handedness.

This presentation will impact the forensic community by examining the statistical validity of using joint asymmetry as an estimation of handedness.

In order to develop a comprehensive biological profile, forensic anthropologists depend on the ability to identify skeletal characteristics. Determining handedness from skeletal material is one tool that would enhance the effectiveness of the biological profile. While bilateral asymmetry has traditionally been used as an indicator of handedness, the statistical significance of this practice needs to be examined. This research examined the statistical significance of bilateral joint asymmetry as an indication of handedness or hand dominance.

Bilateral asymmetry of the long bones has historically been used as a possible indicator of handedness. Longer right humeri were thought to indicate right handed dominance as were longer left lower limbs (specifically the tibiae). Differential use was assumed to manifest in bilateral asymmetry. Kerley (1972) also indicated that the clavicles could be used in handedness determinations. In 2007, the statistical significance of length and weight asymmetry was examined using a skeletal sample of known handedness. Results indicated that asymmetry existed in the sample; however, only the clavicles were clearly correlated with handedness. In 2004, Plochocki examined differential loading of the joints as a result of differential hand dominance; he concluded that environmental activity does result in asymmetry; however, the handedness was unknown for the individuals in Plochocki's sample. In an effort to further examine the relationship between bilateral asymmetry, joint asymmetry, and handedness, the current study examined the proximal and distal epiphyses of the humeri, femora and tibiae of a sample with known handedness.

The William M. Bass Donated Skeletal Collection housed at the University of Tennessee in Knoxville was utilized for the 2007 study as well as for this study. Whenever possible, a biological questionnaire is completed when an individual is donated. These include a question related to handedness. This information in conjunction with the skeletal remains presented a good opportunity to examine the actual statistical correlation between handedness and bilateral asymmetry.

For this study, the humeral head diameter, humeral epicondylar breadth, femoral head diameter, femoral epicondylar breadth, tibial proximal epiphyseal breadth, and tibial distal epiphyseal breadth were measured. The measurements of 107 individual donations with known (self-reported) handedness were examined. When the sample was pooled, paired t-tests of each individual's measurements indicated significant ($\alpha$ =0.05) asymmetry *sans* the femoral head diameter and distal tibial epiphyseal breadth. When the samples were grouped by handedness, the lefties were only asymmetrical in the humeral epicondylar breadth and proximal tibial epiphyseal breadth while the righties were asymmetrical in the same measurements as the pooled sample. Statistically significant differences favored the right skeletal element in each case. Therefore, when both groups were asymmetrical, they were asymmetrical in the same direction. This was not particularly supportive of the handedness-use hypothesis.

While asymmetrical significance was difficult to tease apart, the results of discriminant analyses testing the six measurements' classification ability were promising. Preliminary results indicated that discriminant analyses correctly classified 74% of individuals into handedness groups when utilizing all of the measurements. When the groups were separated by sex, the classification rates improved to 88% for females and 80% for males. The percentage of directional asymmetry {DA = (right-left)/(average of left and right) *100 } was also calculated for each individual. The results of these calculations supported those calculated using the paired t-tests.

Significant bilateral asymmetry is present, but simply using one measurement fails to adequately classify an individual. However, preliminary classification results using all of the measurements are cautiously optimistic. Of note, there is a sampling bias that is present in the sample which may be the cause of apparently conflicting results. Of the 107 individuals, only 15 individuals were classified as left handed; however, this ratio does mimic that found in the general population.

**Bilateral Asymmetry, Handedness, Joints**

## H79    Forensic Anthropology Academic and Employment Trends

*Gina M. Agostini, BS\*, and Emily J. Gomez, BA, 4500 Manor Village, Apartment 316, Raleigh, NC 27612*

The goal of this presentation is to offer valuable insight into the current trends of the profession, to students seeking higher education in forensic anthropology, and to professionals in the field.

This presentation will impact the forensic community by examining employment and academic trends directly affecting the future of the field.

The goal of this project was twofold:  (1) to evaluate current physical anthropology graduate programs affiliated with AAFS members, and (2) to evaluate the type and frequency of employment opportunities available in forensic anthropology.

For the first objective, 41 anthropology programs were evaluated. Forensic science programs including those with an anthropologist on staff were not evaluated as they are under the purview of the Forensic Science Education Program Accreditation Commission (FEPAC).  This project was accomplished with assistance from members of the AAFS Physical Anthropology Section Ad-Hoc Education Committee.  Of these 46 programs, 32 were highlighted as having a learning environment best-suited to forensic anthropology students.  Criteria for recommendation included the availability of a graduate program in anthropology, presence of an AAFS member on staff who is available to serve as mentor, and availability of a specified Master's or PhD concentration or degree track in forensic anthropology.  This review process is ongoing based on changes including individual retirement, membership status in the AAFS, and the development of new programs.   The number of recommended programs is expected to change in the future.

For the second objective, members of the AAFS Physical Anthropology section were invited to participate in an anonymous, broad-spectrum survey which addressed key issues concerning academic background, employment, and professional affiliations.   The survey responses were then compiled to assess the range of employment options available to those who specialize in forensic anthropology.   Non-traditional or non-academic opportunities were also included.   The survey encompassed issues seen as relevant to students, educators and professionals.  Topics addressed included the number, size and location of universities staffed with forensic anthropologists, the size of the graduate program offered and any relevant short courses or workshops offered.  Topics regarding professional and educational background of the instructors were also addressed, as well as the extent to which students were included in relevant research and/or presentations.

Sixty-nine percent of AAFS members participated in the survey. Results showed that 54% of those who participated held a doctoral degree, 34% held a MA/MS or MSc degree, and 7% held a BA/BS degree.   Eighty-five percent of all participants held a degree in Anthropology.   Reported employment affiliation included academia (44.5%), medicolegal agencies (19.1%), federal agencies/institutions (12.7%), museums (2.7 %), non-profit/non-governmental (2.7 %), self-employed/consultant (5.5%), and unemployed, retired or students (12.7%).  Of those who had an academic position, 26.9% were assistant professors, 25% were full professors, 19.2% were tenured Associates, 25% were adjunct professors and 3.8% were untenured associates.  Over half of those who participated in the survey reported regularly including students in their research and/or mentored graduate students. Approximately 46% responded that they did not have any graduate students with a concentration in forensic anthropology.  Results also showed that the states with the most forensic anthropologists were California, Hawaii, New York, and Texas.   Thirty-seven percent of survey participants reported earning between $50,000-$75,000 annually, 28.6% between $75,001-$100,000, 16.9% over $100,000, 11.7% between $25,000-$50,000, and 5.2% reported earning less than $20,000.

In addition, a survey was taken of job advertisements posted on the AAPA website from the last six years.  This showed a wide range of career opportunities available to individuals with specialized training in forensic anthropology.  Of 839 job listings, 321 or 42.3% could be filled by someone with training in forensic anthropology. Listings for specialists in physical/biological anthropology were the most prevalent (187), followed by positions in anatomy (82) and forensic anthropology (32). Osteology (14) and skeletal biology (6) positions had the fewest listings. Thirty-four post-doctoral positions were available in forensic anthropology and 72.3% were tenure-track positions.

**Forensic Anthropology, Employment, Education**

## H80    Biology and Culture in the Modern Era: How Cultural Evidence Can Conflict With Forensic Significance

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After attending this presentation, attendees will have seen an extreme case of ankylosing spondylitis.  Attendees, most importantly, will understand that pathology and individual circumstance can help solve a case, but can also be greatly misleading.  This presentation will demonstrate how evidence, circumstance of burial, and burial artifacts must be analyzed in both biological and cultural contexts.

This presentation will impact the forensic community by demonstrating the importance of the cultural assessment of evidence, determining forensic significance in the face of conflicting evidence, and the presentation of a rare and extreme case of ankylosing spondylitis.

One of the responsibilities of a forensic anthropologist is to determine the forensic significance of unknown human remains that are presented to the Office of the Medical Examiner.   Often times the biological conditions and cultural circumstances of the case can be misleading.  The analysis of evidence, circumstance of burial and burial artifacts must be done in both biological and cultural contexts and is required to understand whether a forensic case falls into the purview of the medical examiner.  In the case described below, the remains were recovered from a shallow grave near Flagstaff, Arizona.  The Federal Bureau of Investigation (FBI) had concerns that the grave might be clandestine and an attempt to hide a homicide.  The recovery of the remains followed standard forensic protocols and revealed interesting clues to the identity of the individual.

In April 2005, the FBI in Coconino County, AZ recovered skeletonized human remains from a shallow grave.  The remains were consistent with those of an adult male of Asian ancestry (including Native American ancestry) and appearing to be 40-55 years old at the time of his death.  Artifacts including a pair of black, lace top boots, two clear colored bottles, and a silver colored spoon were also submitted. The analysis of the decedent revealed that he had an advanced and severe case of ankylosing spondylitis.

Ankylosing spondylitis is a chronic disease that causes inflammation of the joints between the vertebrae and the joints between the spine and the pelvic girdle. Over time, if it progresses, it will eventually cause the affected vertebrae to fuse by means of a layer of additional bone connecting the vertebrae.  This condition is sometimes referred to as dripping candle wax because of its appearance.  There is no known cause of ankylosing spondylitis, but heredity seems to play a role. The disease most frequently begins between ages 20 and 40, but can initiate before age 10.  It affects more males than females and the risk factors include a family history of ankylosing spondylitis and male gender.  Some symptoms include hip and back pain that may begin in the

sacroiliac joints and involve all or part of the vertebral column, chronic stooping, fatigue, joint pain and joint swelling in the shoulders, knees, and ankles, limited expansion of the chest, and limited range of motion (especially involving spine and pelvic girdle).

The location and manner of the individual's burial prompted the FBI to treat this case as a possible homicide. The artifacts and some of the biological evidence, at first, led the forensic anthropologist to deem the case historic. However, when dealing with Native Americans living on or near the reservation, it is imperative to consider that Native Americans lived in "historic" conditions well into the modern age. It is the forensic anthropologist's agenda to consider all evidence in an appropriate manner in order to determine forensic significance. The authors will present literature and photographs of the decedent and the evidence recovered from the grave to illustrate the conflicting evidence and the extent of the disease in this individual and to give participants in the meeting the opportunity to examine an advanced case of the disease.

**Ankylosing Spondylitis, Biology vs. Culture, Forensic Significance**

## H81 Analysis of Thirty-Three Years of Forensic Anthropology Casework at California State University, Chico (1975-2008)

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The goal of this presentation is to summarize and assess statistical patterns in the forensic anthropological cases examined at California State University, Chico (CSUC) from January 1975 to April 2008.

This presentation will impact the forensic community by providing demographic information on cases examined at the CSUC Human Identification Laboratory (CSUC-HIL) that will aid in evaluating trends in forensic anthropological casework in northern California. This study will provide comparative data for evaluating temporal and regional trends in forensic casework in North America, and also will shed light on the changing applications within forensic anthropology since the 1970s.

The research is modeled after a similar study conducted at the Smithsonian Institution (Grisbaum and Ubelaker 2001). Case reports spanning 1975 to 2008 were examined, and a total of 319 cases were entered into the project database. For each case, the date of arrival, anthropologist, trauma type, age, sex, ancestry, and MNI were recorded. Cases were categorized as representing human or non-human remains, or both.

Of the 319 cases recorded, 18.8% (n=60) were non-human or included non-human remains. Of the 264 human remains, 18.2% (n=48) were positively identified through efforts of the CSUC-HIL. Biological profiles were determined on an individual basis, when possible, as several of the cases were determined to have an MNI greater than one. These cases typically involved buried remains (e.g., archaeological), in which commingling was a hindrance to creating individual biological profiles.

A MNI of 328 was determined for the 264 cases that were determined to be human remains. Of these 328 individuals, 43.3 % (n=142) were male and 28.9% (n=95) were female. In the remaining 91 individuals, sex could not be determined due to the incomplete or fragmentary nature of the remains. Of the 328 individuals analyzed, 10.4% (n=34) represented subadults.

The frequency and regional distribution of casework were also analyzed. The majority of cases are from five northern California counties: Shasta (13.2%), Butte (9.1%), Tehama (8.2%), Nevada (6.6%) and El Dorado (6.6%). The remaining cases came from one of 27 additional counties in California or from Nevada.

The extensive data set from CSUC (n=319) will provide an interesting temporal and regional perspective for comparison to other studies, specifically: Hrdlicka's 37 cases from 1938-1942, Stewart's 167 cases from 1946-1969, Angel's 646 cases from 1962-1986, and Ubelaker's 667 cases from 1975-2000 (Ubelaker 2000). At CSUC, the number of case reports of remains determined to be human and non-human were examined by decade. Nonhuman remains comprised 28.6% of cases in the 1970s, although this includes only two cases. In the decades following, the percentage of nonhuman cases increased from 13.3% (n=4) in the 1980s, to 18.8% (n=26) and 20.6% (n=28) for the 1990s and 2000s, respectively. The frequency of human cases increased dramatically from five cases in 1970s to 108 cases from 2000-2008.

This study provides an assessment and summary of the casework conducted at the CSUC-HIL, which will serve as a basis for comparison with other regions of North America. The results of this study indicate a dramatic increase in the use of forensic anthropologists in Northern California beginning in the 1990s.
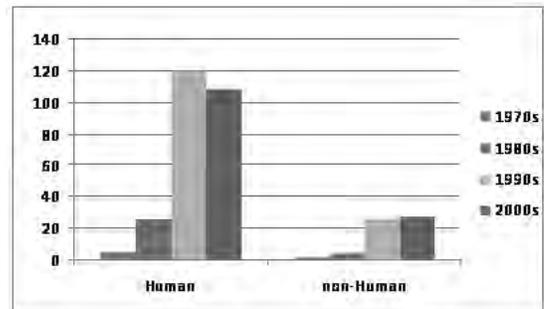
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**Demographic Analysis, Biological Profile, Northern California**

## H82 Detecting Buried Metallic Weapons in a Controlled Setting Using a Conductivity Meter

*Charles A. Dionne, BS\*, University of Central Florida, Department of Anthropology, 4000 Central Florida Boulevard, Orlando, FL 32816*

The goal of this presentation is to introduce the attendees to the utility of the conductivity meter as a geophysical instrument used by forensic investigation teams in the search for buried weapons. Participants will gain a better understanding of how law enforcement agencies can benefit from the use of a conductivity meter when searching for buried metallic weapons.

This presentation will impact the forensic community by demonstrating how the use of geophysical technologies by law enforcement for evidence searches is becoming more frequent. The field of forensic archeology has proven important for the improvement of forensic searches by performing controlled research with various geophysical technologies. By conducting controlled research, it is possible to test the applicability of geophysical instruments during

searches for buried evidence at potential crime scenes or areas of disposal.

This research project will involve the use of a conductivity meter to test the applicability of this instrument to detect buried metallic weapons. Overall, this project is one aspect of a larger project which includes multiple geophysical tools and tests their ability to detect buried metallic weapons. This presentation will solely focus on introducing the attendees to the conductivity meter and to the extent of its applicability for buried weapon detection. The overall objectives of the research were to: (1) investigate the capability of the conductivity meter to detect weapons of various metallic components and sizes at depths up to approximately 75cm, and (2) to create some guidelines on the proper use of a conductivity meter in a forensic setting.

The conductivity meter chosen for this research was the portable Geonics EM38. This model is approximately 1m in length, has a maximum detection depth of 1.5m, and can provide preliminary results in the field. For purposes of this research project, the conductivity readings were recorded while the instrument was on the ground by pressing a trigger button on the handle. The conductivity values were saved using a portable data logger attached to the conductivity meter and were first uploaded into data management software provided by Geonics Limited. Next, the conductivity data was imported into Golden Software's Surfer 8 (version 8.4) for analysis of the conductivity values for each data collection depth, and presentation of the data as contour maps.

A mixture of 32 metallic items was specifically chosen for this research project representing three categories: firearms, miscellaneous metals, and blunt and sharp force objects. The items used include 16 street-level decommissioned firearms (including pistols, revolvers, shotguns, and a rifle) that are constructed from various metals, 6 miscellaneous pieces of metals (including copper and aluminium), and 10 blunt or sharp force weapons (such as a hammer and a machete). The weapons were chosen to represent an assortment of various sizes and compositions. The research site was located in a flat and grassy area that was regularly mowed. The items were buried in 7 rows over a data collection grid measuring 15 by 19m. The size of the grid ensured that the weapons were evenly spaced with enough distance between each to avoid false results. Each burial hole was marked with an orange, plastic stake to easily identify each burial location. The conductivity values were obtained by recording data every 25cm along transects also separated by 25cm. Conductivity data was first collected when the weapons were buried at a depth of 30-35cm. The weapons were then reburied 5cm deeper each time data collection was performed down to a maximum depth of approximately 75cm.

Initial results show that the conductivity meter is able to detect multiple targets from all 3 categories at several depths. For example, at a depth of 45-50cm, 10 of the 16 firearms, 2 of the 6 miscellaneous metals, and 6 out of 10 blunt or sharp force items were detected. The items that were not detected were mostly the smallest items in the grid such as the small revolvers, the copper tube, the piece of rebar, the knife, the screwdriver, and the brass knuckles. At deeper depths, the conductivity meter was still obtaining strong signals from the larger weapons such as the shotguns, the rifle, the machete, the hammer, the mallet, the prybar, the scissors, and some of the larger hand guns such as the magnum and the Ruger. It is also important to note that the control hole was never detected and therefore the positive results were the result of the detected weapons and not the disturbed soil. Overall, the conductivity meter provided strong results at all depths tested with large weapons and would be a viable geophysical instrument for law enforcement agencies that are searching for large hand guns, shotguns, or rifles.

**Forensic Archeology, Conductivity, Controlled Research**

# H83 The Effects of Ethanol Abuse on Bone Mineral Density in the Proximal Femur

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After attending this presentation, attendees will have a better understanding of secondary osteoporosis and the relationship between bone mineral density and alcoholism.

This presentation will impact the forensic community by explaining that the results of this study using ANOVA showed no significant difference between the BMD of ethanol abusers and the control group. The authors conclude that it is preemptive at best to state whether or not a skeleton with a younger age and bone de-mineralization was possibly alcoholic.

This research explores the effects of alcoholism on bone mineral density of the proximal femur using dual energy X-ray absorptiometry (DEXA). The clinical consequences of excessive alcohol consumption have been well documented in the medical literature. The goal of this research is to test the assumption that bones from a sample of known ethanol abusers will have reduced bone density compared to an age-matched control sample.

Due to confounding effects of alcohol related illnesses, research evaluating the changes in bone density related to chronic ethanolism has been limited. Moreover, population-based analyses of alcohol-related bone loss are hindered by a lack of control for patient ages, sex and age specific hormonal effects, variability in the duration and patterns of alcohol abuse, and questionable self-reporting, which yield obscured and contradictory results.

Purportedly, it is common knowledge among forensic anthropologists that chronic alcoholics frequently present characteristics similar to those observed in individuals of advanced age or "osteoporotic" skeletons. This information is often inherited from mentors or practically acquired while working with skeletal collections for which medical history is reported or in similar "known" contexts. Alcoholism has received little attention in discussions of forensic anthropological method and theory. A recent literature review of AJPA and JFS shows an absence of empirical investigation and published documentations. As a result, suggestions of alcoholism are commonly relegated to side notes (*possible alcoholic?*). For example, the assessed age indicators do not warrant an assignment of "old age" for the unknown individual and do not support age-related diagnosis for the skeletal demineralization, especially if pathological explanations are not logical.

The sample consists of the femora of 35 white males from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville between the ages of 20 and 70 years old. Fifteen of the males reportedly suffered from alcoholism. This collection offers a unique opportunity to study skeletons known individuals with medical histories. Only males were sampled in order to reduce the effect of sex-specific risks typically found in the female skeleton. Due to the small sample of ethanol abusers, the control sample is age-matched. DEXA is a simple and inexpensive means of establishing bone mineral content (BMC) and bone mineral density (BMD = BMC/area) at different regions of the body. Each femur was placed in a plastic container filled with dry white rice to a depth of approximately 12 cm. The rice served as a soft-tissue density equivalent for the DEXA scans, as suggested by GE, the producer of the DEXA Lunar scanner. A 2 cm thick cube of low-density foam was placed under the lesser trochanter to approximate anatomical position. Standard measurements of bone mineral density

(BMD)(g/cm2) were calculated automatically for the femoral neck, Ward's triangle, the greater trochanter, proximal shaft and total BMD.

The results of this study using ANOVA showed no significant difference between the BMD of ethanol abusers and the control group. The authors conclude that it is preemptive at best to state whether or not a skeleton with a younger age and bone de-mineralization was possibly alcoholic. There exist too many confounding factors related to chronic alcohol abuse to clearly state whether loss of bone mineral density is the direct result. Confounding variables include the effects of body mass, nutritional deficiencies and hormone balance, among others. For clinical applications, it is important to predict osteoporotic fracture and for the development of orthopedic devices. Bone density analysis may be of interest to forensic anthropology and bioarcheology in order to individuate human skeletal remains for other purposes, but low BMD does not conclusively suggest alcoholism in the current study.

**Alcoholism, Bone Mineral Density, DEXA**

## H84 Geophysical Remote Sensing Applied to the Forensic Search for WWII Graves in Guadalcanal

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After attending this presentation, attendees will have a better understanding of the use of selected remote sensing techniques (Ground Penetrating Radar and a capacitively coupled resistivity system) applied to the search for clandestine graves, including those from historic 19th and 20th century Virginia as well as covert mass graves from WWII in Guadalcanal, Solomon Islands. Recommendations for future forensic searches will be provided.

This presentation will impact the forensic community by demonstrating remote sensing investigation of clandestine mass graves in a variety of diverse environmental and temporal periods. Advantages and limitations of these techniques in specific burial environments (e.g., Central and Eastern Virginia and Guadalcanal, Solomon Islands) and 19th and 20th century temporal contexts are highlighted, as are applications of these techniques in multidisciplinary forensic investigations.

Geophysical remote sensing techniques have been applied to the forensic search for clandestine graves for more than 15 years. Recently, Schultz[1] has cited the need for additional controlled remote sensing research to elucidate the effectiveness of these techniques in different micro-environments and longer interment periods. The current research does just that, by applying Ground Penetrating Radar (GPR) and a capacitively coupled resistivity system (i.e., Ohm Mapper) to mid-19th century and mid-20th century sandy and clay as well as wet and dry burial contexts in both the northern and southern hemispheres.

The ill-fated U.S. Marine Goettge Patrol was ambushed by the Japanese on the evening of August 12, 1942, in Guadalcanal, Solomon Islands. Only three of the patrol survived; Japanese accounts indicate that 17 of the dead Marines, along with some dead Japanese soldiers, were buried in a group of Japanese rifle trenches. Their remains have never been recovered, although there is much speculation about the probable location of their interment.

In July, 2008, an interdisciplinary team of forensic anthropologists, archaeologists, historians, and physicists conducted the Goettge Patrol

Guadalcanal survey. The goal of this survey was to locate the Japanese defensive trenches where members of the patrol were supposedly buried by using geophysical remote sensing equipment (GPR, Ohm Mapper), followed by archaeological test excavations of the identified anomalies. Two GPR systems were used: the Noggin Plus 250 Smartcart with a 250 MHz antenna and the Pulse Ekko GPR system with both 100 MHz and 500 MHz antennae. The Ekko Mapper 3 and Voxler software programs were used to visualize and process the data. Nearly 7,000 square meters of the most likely target area (based on historical and archival research) were surveyed. In addition, a capacitively coupled resistivity system was used to survey a 2,400 square meter area of high burial probability (also surveyed by the GPR), which, when processed using Mag Map 2000, produced a three-dimensional plan view of the data.

Anomalies identified through these remote sensing techniques were then tested by hand excavation of standard archaeological test units and trenches. Although the clandestine graves of the Goettge Patrol were not identified, interpretation of the anomalies was instructive in understanding the history and soil stratigraphy of the site and the impact of natural and human factors on the area since WWII (e.g., ground water accumulation and depositional fill). These investigations also helped narrow the search area in terms of where the Goettge Patrol is not.

The Guadalcanal remote sensing survey highlighted the complexities of using remote sensing techniques in sandy, wet soil and extended interment periods. Further elucidation of these variables was accomplished by applying the same technology to 19th and 20th century historic graves in Arlington, Virginia and at the R.J. Reynolds Homestead in Critz, Virginia. From these investigations, it was learned that identification of graves of considerable postmortem interval was possible, but necessitated the use of a multidisciplinary team approach integrating the specialized skills of archaeologists, forensic anthropologists, physicists, and historians.

**Reference:**
[1]  Schultz J. Sequential monitoring of burials containing small pig cadavers using ground penetrating radar. *JFS* 2008; 53; 279-287.

**Forensic Archeology, Remote Sensing, Clandestine Graves**

## H85 Necessary Breaks With Conservator Standards: Cranial Reconstruction in Forensic Cases

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After attending this presentation, attendees will have a better understanding of the advantages of various methods used in the reconstruction of fragmentary skulls and teeth, when particular techniques are appropriate, and solutions to common problems that result from plastic deformation, missing fragments and taphonomy.

This presentation will impact the forensic community by differentiating between processes that are better suited for forensic settings as opposed to museum/conservation contexts. Special attention to also paid to adhesive variables, such as pH, solubility, flammability, tensile/shear strength, and reactivity/stability.

Most protocols used in skeletal reconstructions are rooted in methods created for museum curation and conservation laboratories where bone stabilization, preservation, aesthetic appeal, and future study are emphasized while timeliness and cost are less important. These latter

two factors are extremely vital to forensic investigations, however, where funding is limited and time is in short supply. Also in forensic settings and in contrast to museum/conservation objectives, the completed skeletal reconstruction does not need to be indefinitely stable and destructive procedures are often acceptable. As such, forensic practitioners have broken from some museum/conservation standards while still maintaining many of the basic procedures necessary to ensure that osteological analyses lead to victim identifications and establishing a profile of trauma. To this end, the step-by-step forensic process is presented for use in the successful reconstruction of cranial bones and teeth that have been fragmented from peri- and/or postmortem trauma.

This survey of conservator and forensic methods found that prior to cleaning and reconstruction, the standard procedures were markedly similar and included: the documentation of the fragmentary bones *in situ* (e.g., at the scene, transport containers, or soil matrix), radiography of the materials before processing, and localized evaluation of the bone to ensure it was structurally sound and therefore could withstand cleaning. Conservator and forensic methods also differed when the process of cleaning was surveyed. Cold water maceration, hot water maceration, and dry brushing were successful. Strong chemical regents (e.g., hydrochloric acid, hydrogen peroxide, ethyl alcohol, methylated spirits, household degreaser, or household detergents) were rarely used. While this method of cleansing the bone was fast, cost effective, and did not tend to warp the bone, the bones remained slightly greasy and fatty thereby requiring suitable adhesives for reconstruction.

Acid-free adhesive composed mostly of acetone and nitrocellulose (e.g., Duco™ cement) was ideal for forensic casework because it set quickly, was easily reversible (with acetone), and had moderate adhesive strength. On the contrary, conservators tended to use Acryloid B-72 or Polyvinyl Acetate (PVA) because long term strength and preservation was the main objective. Other factors that were considered when choosing an adhesive included: pH level, stability, solubility, and flammability. An adhesive that was pH neutral (or acid free) was imperative as an acidic or alkaline adhesive would damage bone. Selecting an adhesive that was stable under temperature and humidity fluctuations was also necessary in forensic contexts, but less important in museums, as humidity and temperatures were strictly controlled. Because forensic remains often retain some internal moisture through grease or fat, an ideal adhesive should be insoluble in water. Due to the adhesive properties necessary in forensic contexts, obtaining a flammable adhesive was sometimes unavoidable. However, practitioners should use caution and be aware of their adhesive's flammability rating.

Several cases are presented that sustained perimortem fractures via gunshot wound, burning, or blunt force trauma. Postmortem fracturing from removal of the calotte and jaw during autopsy was also present. Issues that arose from these types of trauma included disassociation of the teeth from their crypts, plastic deformation (e.g., a series of microfractures that causes warping), bone loss, and delamination. In order to ensure accurate cranial measurements and trauma analysis the vault and face were reconstructed separately. In addition, the vault and face were not fixed together with glue. Rather, inert dental wax was used allowing the calotte, basicranium or splanchnocranium to be moved in order to ensure the accuracy of the various measurements. In addition, replacing bone with dental wax along autopsy dissection lines increased our accuracy as the reciprocating saws removed from 2mm to 8mm of bone. As cases in point, this study presents examples in which classification of sex, ancestry, wound diameters, and numbers of impact sites changed based upon the reconstruction processes used.

**Anthropology, Fragmentary Remains, Reconstruction**

## H86 Anthropology for Breakfast: A Semi-Cautionary Tale

*John A. Williams, PhD\*, Anthropology & Sociology, Western Carolina University, 101 McKee Hall, Cullowhee, NC 28723*

After attending this presentation, attendees will have an understanding of the triage and decision making process that forensic anthropologists experience when confronted with an atypical item.

This presentation will impact the forensic community by demonstrating why forensic specialists should be consulted, no matter how mundane the case may appear.

Forensic anthropologists routinely are asked to identify non-human bone, and sometimes items that are not bone at all.

A graduate student at a southeastern university made, what to her was, a disturbing discovery in her breakfast cereal. A store brand box of raisin bran contained an item that at initial observation appeared to be a piece of bone with dried, attached flesh and connective tissue. For reasons never stated the student approached the biology department of the university and asked for an identification of the unknown object. (It is surmised that legal action would be taken should the item be identified as animal in origin.) The biologist she contacted is a microbiologist with no hard or soft tissue anatomical background. Despite this, he confirmed that the item was some form of animal anatomy. Fortunately, the biology professor suggested that the student get a second opinion from a forensic anthropologist.

The errant breakfast food item was brought to a forensic anthropologist at the university. After relaying the story of the discovery, the forensic anthropologist made a gross macroscopic examination of the item. The item was small (18.5 mm x 7.8 mm x 5.0 mm) and slightly L-shaped. The exterior texture was soft but not sticky. The visual appearance did strongly resemble a piece of dried flesh, not unlike jerky. The item, however, failed the first basic test, the "sniff test." It did not smell like flesh, if anything the smell was neutral. This led to the conclusion that the item was not likely animal in origin.

To confirm this initial conclusion, permission was obtained to examine the item in more depth. Only limited dissection was granted by the student, however. Using a Keyence digital microscope the item was examined thoroughly at various levels of magnification. Using a scalpel, a small area was dissected. All in all, the item had the appearance of dried or semi-dried flesh complete with dried blood. Digital x-rays were taken using a Faxitron unit to examine the internal structure. The radiographs indicated that the central core of the item had a different density than the outer surface. This inner core, however, was uniform in density and did not display the compact-cancellous bone contrast expected of bone. To confirm this, a rodent femur and a tree stem of similar size were also radiographed. The rodent femur had the radiographic signature of bone. The tree branch, like the unidentified item, displayed a uniform density with little distinction between the outer and inner surfaces.

With this information it was concluded that the item was a piece of vegetative matter. It is likely a piece of grape vine with crushed red raisins adhering to the outer surface. No doubt "quality control" at the cereal factory was not at peak performance. The student was assured that there was no biological hazard created by the presence of this item in the cereal.

Despite the knowledge of a forensic anthropologist on campus the biologist with no anatomical background made a determination as to the origin of this item. This is a dilemma forensic anthropologists continue to face; non-anthropologists (and in some cases anthropologists) stepping beyond their training and expertise. Fortunately for all involved, the correct origin of the item was ascertained, avoiding no doubt, later embarrassment. It is not known whether a new box of cereal was provided as a result of this discovery.

**Triage, Identification, Non-Human**

## H87 Unusual Skeletal Variations Observed in an Adult Aboriginal Male: Case Study from Brisbane, Australia

*Donna M. MacGregor, MSc*, Queensland Police Service, Scientific Section, 200 Roma Street, Brisbane, 4001, AUSTRALIA*

After attending this presentation, attendees will understand the role of police crime scene examiners in Queensland with relation to skeletal recovery and the unique role they serve in Coronial investigations. Attendees will also understand that standard age techniques were unable in this instance to assist with determining a reasonable age range for this individual.

This presentation will impact the forensic community by illustrating the unique role of police officers and routine variations encountered during skeletal casework within Queensland, Australia.

On July 1, 2007, forensic scientists from the Queensland Police Service Physical Evidence Unit went to a bushland location within an industrial area approximately 18 kilometers southwest of Brisbane, Australia in response to the discovery of human remains. The remains were located on a vacant block of land surrounded by a heavy industrial area. This area was routinely used by truck drivers to park heavy vehicles. The human remains were completely skeletonized, devoid of tissue, and had associated items of clothing. The remains were located within a small area and exhibited limited scattering effects by local predators. Flyscreen spline containing cervical vertebrae looped around a tree was also located. A humpy (small, temporary shelter) was located near the site. Almost all of the skeletal elements were recovered. This included most of the long bones, disarticulated pelvis, cranium and mandible, ribs, vertebral bodies, sternum, thyroid cartilage, and hyoid bone. All of the teeth were also present.

On the 3rd of July 2007, a forensic osteologist from the Queensland Police Service went to the Queensland Health Forensic & Scientific Services morgue to assist with the postmortem examination of the skeletal remains. The remains were consistent with an Aborignal male with an approximate living stature of 173 +/- 3.91cm. The determination of age was difficult using traditional aging techniques. All long bones had fused. The sternoclavicular joint had fused. The thyroid cartilage was almost completely ossified and consistent with Stage 8 of Vleck's method corresponding to an range of 51-58 years. However, the pubic symphysis was consistent with the Suchey-Brooks method Phase II suggesting an age range of 19-34 (95%). Extensive pathology and a wire was observed within the sternum and manubrium indicating previous thoracic surgery. The age range given to investigators at the time of the autopsy was 25-58 years at the time of death.

The details of the biological profile obtained during the autopsy were supplied to the investigators to compare against the Missing Person Database. No links were obtained, and therefore no possible identity was forthcoming.

During subsequent examinations of the recovered clothing, deterioated paperwork was located within an item of clothing. This paperwork provided crucial information which aided in the identification process. These documents included a bail conditions release form with a name believed to be that of the deceased. DNA taken at the time of autopsy confirmed the identity of the remains. The deceased was 27 years of age. It was discovered that the deceased had been involved in a stabbing incident two years earlier and had undergone thoracic surgery. Also of interest was that no missing person reports had been lodged for this person. A search of police computer systems revealed that the deceased had been last involved with police approximately 8 months earlier.

**Forensic Osteology, Age Estimates, Scene Recovery**

## H88 Estimation of Age at Death From the Juvenile Scapula

*Natalie Uhl, MS*, 308 North Orchard Avenue, Apartment 7, Urbana, IL 68101*

After attending this presentation, attendees will understand a method for estimating age at death from the juvenile scapula.

This presentation will impact the forensic community by presenting a method for age at death estimation that can be utilized when juvenile remains are incomplete or fragmented.

Age estimation in juveniles has traditionally focused on long bone lengths, epiphyseal union, and dental development, but when presented with fragmented or partial remains, other skeletal elements must be employed. Rissech and Black (2007) included an age estimation method that uses scapular measurements as a part of a broader study on the development of the scapula in juveniles. In that study, the authors utilized nine measurements and polynomial regression to explore and explain the growth of the scapula from birth to sexual maturity in a sample of 31 juveniles drawn from the Scheuer Collection housed at the University of Dundee. In order to estimate the age-at-death of an unknown individual, the Rissech and Black study presents eight regression equations derived from inverse functions of a simple linear regression. A ninth equation utilizing the measurement "acromial width" uses the inverse of a second order polynomial regression for age estimation. Because the study sample includes individuals past the age of sexual maturity each of the nine equations falls into one of two groups. The first group of equations is for individuals before the development of secondary sexual characteristics while the second group is for older individuals. Although it may seem appropriate to consider the sex of the individual, particularly in older individuals, their study pools males and females of all ages.

The purpose of the current study is to test the applicability of the equations published by Rissech and Black (2007) for estimating age-at-death in a sample of 19th century Americans. The study sample consists of 40 individuals (19 males, 21 females) ranging from 0 to 22 years of age (mean = 9.9 years) from the Hamann-Todd Collection housed at the Cleveland Museum of Natural History in Cleveland, Ohio.

Acquisition of the nine scapular measurements was fairly straightforward, with the exception of "glenoid mass" which is difficult to measure after age 16 or 17 when the coracoid process fuses to the superior margin of the glenoid. The results of the Rissech and Black study include positive correlations between actual and estimated age that range from 0.78 to 0.91. The results from this study were comparable to their findings, with correlations ranging from 0.83 to 0.92. Bias statistics indicate each equation is underaging the individuals on average, but the low inaccuracy values indicate that the overall estimates are accurate.

The Rissech-Black method of juvenile age estimation is simple and applicable. This test indicates that their equations are highly correlated with age. While correlations are not necessarily the most appropriate way to judge the effectiveness of an aging method, highly positive correlations do indicate that the scapula can provide important information pertaining to age estimation. Long bone length, epiphyseal union, and dental development are generally the aging indicators of choice when dealing with juvenile remains, but this method offers an alternative for anthropologists when faced with fragmented or incomplete remains.

**Age Estimation, Juveniles, Scapula**

## H89    Forensic Age Estimation of Living Individuals:  A Retrospective Case Analysis

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The goal of this presentation is to demonstrate that it is necessary to standardize the methods used in the age estimation of living subjects, and that prudence is also necessary in the approach to age determination.

This presentation will impact the forensic community by demonstrating the necessity of formal training in this area.

Age estimation of living individuals undergoing criminal proceedings or requesting asylum is one of the most difficult problems in forensic medicine.  It is a prerequisite for personal identification and it is increasingly important in criminal matters.  In fact, if doubts arise regarding the age of a person suspected of a crime, forensic age estimation is promptly requested by authorities to ascertain whether the person concerned has reached the age of imputability (criminal responsibility).  On the other hand, if an asylum seeker is a minor, his request must be granted.

In Italy, the age limit for which a person is considered to have legal responsibility is 14 years, and the age to determine if an individual should be tried under juvenile delinquency laws or general criminal laws is 18 years.

Particularly between the ages of 14 and 18 years, juvenile delinquency laws in Italy may be applied and it is possible for children to be incarcerated in juvenile detention centers if their responsibility for a crime is determined.  The need for accurate techniques of age estimation has never been greater than it has been in the last two decades due to the increase in international migration.  Most young immigrants have no valid proof of their date of birth (legal age) upon arrival in this country.  They usually lack valid identification documents and often provide an incorrect age.

This study is a retrospective review of a sample of 53 immigrants (32 males and 11 females) coming from two geographic areas which are centers of important world conflicts: the Balkan Peninsula (Slovenia, Belgrade, Zagreb, Albania) and the Middle East (Palestine, Lebanon, Iraq, Egypt).

In these particular cases, forensic age estimation was requested of the Department of Legal Medicine at the University of Bari by local authorities in order to either ascertain criminal responsibility, provide temporary shelter, and/or diplomatic asylum.  The period in question is from May 1989 through September 2007.

The age estimation process began with a clinical examination of the individuals which consisted of recording the stature and weight of the individual, along with a description of his or her secondary sex characteristics, such as the presence of pubic and underarm hair, the development of the external genitalia, and breast development. With the aim of making an age estimate as precise as possible, ortopantomographs, and x-rays of the left hand and wrist on almost every subject were carried out in order to evaluate skeletal and dental age.  In 23 cases, pelvic x-rays were taken, and in a few other cases supplementary examinations were performed (vertebral x-rays, knee x-rays, and shoulder x-rays).

In the retrospective analysis of a sample of 53 subjects, 22 of these demonstrated a possible correspondence between the declared age and the biological age obtained through evaluation by the specialists. However, there was no correspondence for the other cases.  In 11 cases the declared age was less than or equal to 14 years, and only 5 of these 11 cases were confirmed by radiographic examination (OPG and hand/wrist X-rays).

Similarly, there were only 27 cases in which the declared age and biological age corresponded.  Another interesting finding was the correspondence between the results obtained from the analysis of the x-rays of the left hand and wrist and the results obtained by the analysis of the ortopantomographs in almost all subjects.  Statistical analysis was performed and the results showed a significant statistical difference between declared age and the assessed biological age ($p<0.001$); whereas, no statistically significant differences were found between the assessed skeletal and dental ages ($p=0.431$).

It is necessary to standardize the methods used in the age estimation of living subjects, and that prudence is also necessary in the approach to age determination.  Very few physicians are skilled in forensic age estimation procedures either because they have no formal training in this area or, when they do have training, they do not perform these procedures often enough to keep up to date.  In order to correctly estimate age in living subjects, the margin of error associated with the forensic methods adopted must be taken into consideration.

**Age Estimation, Personal Identification, Retrospective Analysis**

## H90    Sealed For Your Protection II:  The Effects of Corrosive Substances on Human Bone and Tissue

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The goal of this presentation is to understand the effects of various common corrosive substances on human bone, teeth, hair, and nails and identify the particular agent used in a recent homicide.

This presentation will impact the forensic community by demonstrating how certain common corrosive agents can affect bone. The forensic community can use the data provided to help identify if and what type of corrosive agents may have been used in an attempt to alter human remains.

In 2006, the remains of a woman and her two children were recovered from 55 gallon drums buried in a desert area west of Phoenix, Arizona.  Based on the condition of the bodies, the use of an unknown corrosive agent to obfuscate identity was suspected.  Multiple white plastic safety seals commonly used to secure containers of corrosive substances were found in conjunction with each of the three drums.  The two children were almost completely consumed by the agent and the adult female had extensive marring of her soft tissue and skeleton consistent with some type of corrosive substance.  Evidence of extensive leaching into the soil suggested a breach of the drum containing the adult female. The large number of safety seals suggested the use of a chemical agent that is easily acquirable.  This study tests a variety of corrosive agents and their effect on human bone and tissue in an attempt to determine the possible agent used.

Six commonly available corrosive substances (muriatic acid, sulphuric acid, household lye, bleach, a 100% natural active bacteria and enzyme product, and a cola soft drink) and a control (tap water) were tested in undiluted form.  Two inch round glass jars were filled with approximately one ounce of liquid or in the case of the dry chemicals, with a mixture of the powder and approximately one ounce of water.

A human male femur was purchased from a medical research company and was sectioned along the shaft.  Soft tissue was removed from the femur.  Cut hair and fingernail clippings were obtained from a salon and pulled teeth were donated from a forensic odontologist.  Each of these specimens (except the fingernails) was weighed using a digital scale accurate to 1/10$^{th}$ of a gram.  The specimens were described and

photographed before being placed into the two inch round jars with the various liquids. Observations were made at specified intervals depending on the agent under consideration. For example, muriatic acid, which is also known as hydrochloric acid, consumed all human tissue types very rapidly so observations were made at half hour intervals until the specimens were no longer present. For those specimens that were not completely consumed, a designation of "no longer recognizable" was assigned. Initially, two runs of muriatic acid were completed to ensure that there was consistency in the observed changes. The results of these two tests were indistinguishable.

The results of the testing varied from consumption of human hair by household lye in three minutes to no observed change in all specimens placed into the natural organism product or water. Muriatic acid consumed all samples (except hair) in 24 hours or less. The rate of loss was steady over the course of the experiment. Sulphuric acid consumed the bone and the tissue over a period of several days while making the bone and tooth soft and viscous. Bleach consumed the hair in 20 minutes, the fingernails in several days and merely whitened the bone and tooth. The cola darkened the bone, nails and tooth but had no measurable effect on any of the specimens. The fingernails turned fluorescent yellow in the lye but the bone and tooth were unaffected.

While some of the substances were effective on individual specimens, the muriatic acid was the most effective across all of the tested material. The result of this experiment suggests that muriatic acid is the most likely agent used in the case described above. Future experiments will include more extensive testing of complete body parts as well as testing human blood and skin with the agents used in this study.

**Corrosive Substances, Human Bone, Human Tissue**

# H91 Aquatic Taphonomy in a Lacustrine Environment: A Case Study From Southeastern Tennessee

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After attending this presentation, attendees will learn about characteristic taphonomic changes brought about after long-term submersion in a lacustrine environment.

This presentation will impact the forensic community by describing a rare taphonomic context and adding to a growing body of literature on the subject of aquatic taphonomy.

In addition to standard components of the biological profile, forensic anthropologists are often called to analyze taphonomic changes of ossified tissue from many types of environments. Though recent developments in forensic taphonomy have described numerous possible alterations to human remains, several types of taphonomic modification are poorly documented by forensic practitioners. For example, few studies have reported findings of skeletal remains recovered from vessels submerged in aquatic environments. The case study presented here describes the condition of one set of skeletal remains recovered from an automobile submerged for over half a century in a lacustrine setting.

On February 22, 1956, a young adult female disappeared from Bradley County, Tennessee. Family and friends had no communication with her after this date and she was subsequently declared dead in 1975. Numerous scenarios surrounding the disappearance were discussed by members of her family and local community; however, none of these hypotheses offered any physical proof as to the missing woman's whereabouts. The case remained unsolved for over fifty years and received little attention until local law enforcement recently dedicated a substantial portion of time to retracing her last known activities.

After the case was reopened, interest soon turned to an alleged car trip taken by the missing woman that February morning. Investigators traced the alleged route along the rural state highway and noticed the roadway's proximity to a 1,900 acre lake. A diving team was assembled to search the lake bottom and subsequently found a vehicle that matched the description of the missing woman's 1951 Chevrolet BelAire approximately 45 feet below the lake surface. Upon searching the interior of the automobile, divers located and recovered numerous skeletal elements and remnants of clothing. All items were then transported to the Forensic Anthropology Laboratory at the Regional Forensic Center in Knoxville, Tennessee for analysis.

After a thorough drying period of several days, available skeletal elements were inventoried and it became clear that the remains of a single individual had been recovered from inside the automobile. The difficult scene prohibited a total recovery, as numerous portions of both the cranial and post-cranial skeleton were missing. It also became apparent that the lacustrine environment had a remarkable taphonomic effect on the skeleton, as numerous elements presented heavy taphonomic alterations.

Taphonomic changes to the skeleton were pronounced. Most notably, portions of the skeleton comprised primarily of trabecular bone were heavily abraded and eroded. In addition, commonly damaged elements such as the scapulae and ribs were heavily fragmented and poorly represented. With the exception of the radii and ulnae, all long bone epiphyses were highly eroded or absent altogether. Of the six recovered vertebrae, none had complete bodies and all but one was represented exclusively by the neural arch. Pubes and ischia were absent while ilia presented a highly weathered appearance akin to long-term sun exposure.

Though hindered by recovery and taphonomy, standard osteometric methods were used to develop the biological profile of the decedent. Sex was estimated from portions of the *os coxae* and metric analyses of the right radius and ulna. These data, along with an overall gracile appearance, led to a female sex diagnosis. Age-at-death was estimated from available portions of the auricular surface. Though portions of the retroauricular area were not present, the absence of billowing and presence of striae produced an age-at-death estimate of 25-34 years. Stature was calculated using the maximum length of the right radius and ulna. Ancestry was not definitively determined as the craniofacial skeleton and cranial base were entirely absent. Though no positive means of identification were possible from the available skeletal elements, available osteological data indicated the missing woman could not be excluded as a possible match. Given that the remains of a young adult female were recovered from the same type of automobile the missing woman drove, and was located in close proximity to a route she was known to travel,, it is argued that a presumptive identification is possible at this time.

**Aquatic Taphonomy, Lacustrine Environment, Forensic Anthropology**

# H92 Recovery of Human Remains From Vehicles Submerged in Fresh Water

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After attending this presentation, attendees will begin to appreciate the unique difficulties and specialized procedures involved in conducting controlled recoveries of decomposed or skeletonized human remains from vehicles submerged in fresh water rivers and lakes. Two recent examples from Indiana are used to illustrate how anthropologists modify traditional techniques of archeological excavation and taphonomic reconstruction to fit these unusual circumstances.

This presentation will impact the forensic community by using two recent examples from Indiana to illustrate how anthropologists modify traditional techniques of archeological excavation and taphonomic reconstruction to fit these unusual circumstances.

The first case involved an automobile submerged in a major western Indiana river. The car was discovered by local authorities and linked to an individual who had been missing for five years. Divers verified the presence of human remains by retrieving a femur through the partially opened driver's side window. The automobile was removed from the river and subsequently stored in a secure evidence bay. The University of Indianapolis Archeology and Forensics Laboratory (AFL) was contacted to assist in recovery of the remains from the car. The members of the AFL team were the first individuals to see the car's interior since its retrieval from the river. Even though this forensic scene was confined to the automobile's interior, it still required thorough and systematic processing. The driver's side window was open approximately six inches and had faced up-river prior to discovery. Large deposits of river sediment had washed into the vehicle, mixing and burying the remains. Pneumatic "jaws of life" were used to open the passenger door, where the sediment was less pronounced. Excavation was conducted using a mix of hand trowels and plastic and wooden tools in order to minimize damage to the bones. Sediment was transferred to a wet-screening station to find small elements. While no formal mapping was conducted, the locations of bones were recorded as discovered, and a field inventory of the remains was conducted throughout the excavation process. The distribution of the individual bones allowed for a reconstruction of the likely original position and location of the decedent at the time of the crash. The body was completely skeletonized and the bones presented taphonomic modifications typical of fresh water interment, including sediment staining and superficial erosion.

The second case involved a jeep submerged in a retaining pond in central Indiana. The vehicle was registered to an individual who had been missing for 1.5 years. It was removed from the water and initial investigation of its interior produced a partial corpse. The individual's postcranial elements were articulated due to the protective nature of the clothing still on the body as well as by thick adipocere formation. The head and hands were fully skeletonized and dispersed throughout the sediment, which had accumulated along the floorboards. Similar recovery techniques were applied in this case, as described above. However, fewer elements were missing and the jeep had an open design that allowed water and sediment to flow more freely without being trapped.

Both cases presented unique challenges and required the anthropologists to adapt and modify their archeological recovery strategies. Facilities and equipment for water screening large amounts of sediment are essential, and the local authorities will have to find a way to dispose of this material. Special safety procedures are required to protect the investigators from sharp, rusty metal, glass fragments, and even tiny fish bones, which can be incredibly sharp. Water action makes it difficult to reconstruct the original position of the body within the vehicle, information that may be essential to understanding the nature of the accident. Lastly, the taphonomic processes that modify human remains submerged in deep water are very different from those that affect remains on the surface or buried in the ground.

**Submerged Vehicles, Human Remains, Forensic Archeology**

## H93 Decomposition Variables: A Comparison of Skeletal Remains Recovered After Long-Term Submersion in Florida Aquatic Environments

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The goal of this presentation is to establish baseline data that will serve to determine postmortem submersion intervals from subaqueous death scenes within Florida's waterways.

This presentation will impact the forensic community by providing medical examiner personnel, law enforcement agencies, and anthropologists with resources that will enable them to collect and analyze data concerning taphonomic variables specific to aquatic submersions. The presentation also demonstrates how these data are essential for the estimation of an accurate postmortem submersion interval (PMSI) by presenting several cases involving aquatic submersion.

Subaqueous death scenes pose myriad investigative challenges including the accurate estimation of postmortem submersion interval (PMSI). The forensic standard in subaqueous PMSI has been broadly defined as one week on the ground's surface equals two weeks in the water—no matter the geographic location. Many specific extrinsic-environmental variables, however, are easily identified and data-mined from water management district environmental station websites that can yield relatively more precise estimation of PMSI specific to the geographic area of interest. To this end, both extrinsic environmental and intrinsic *corpus delicti* data were collected and analyzed to create PMSI for several forensic death scenes within Florida's waterways in order to establish a PMSI baseline for the state.

For this study, the extrinsic environmental variables included ambient and water temperature, salinity, pH level, presence or absence of flora and fauna, depth of immersion, subaqueous substrate (e.g., sand, soil, and cement), bacterial content, algal growth, water current speed, and trace mineral content. These data were easily accessed because of the State of Florida's Water Resource Act which requires water management districts (Northwest Florida, Southwest Florida, St. Johns, Suwannee River, and South Florida) to manage and protect water and related natural resources through monthly (sometimes daily) data collection. As such, most of the extrinsic environmental data was obtained through water management districts' websites (http://www.nwfwmd.state.fl.us/, http://www.swfwmd.state.fl.us/, http://sjr.state.fl.us/, http://www.srwmd.state.fl.us/, http://www.sfwmd.gov/). Additional extrinsic variables included the presence or absence of clothing, footwear, and other personal effects, as well as whether the remains were found within vehicles or other human-made structures.

Intrinsic *corpus* variables included the presence and absence of adipocere, location of adipocere, presence or absence of skin and muscle tissue, location of skin and muscle tissue, organ weights, organ histology, and skeletal inventory and analysis of bone integrity (e.g., cortical bone flaking, trabecular bone wasting, etc.).

The study materials came from forensic cases distributed through several counties across the state of Florida, including Broward, Lake, Lee, and Suwannee counties. For each case, the forensic anthropologist

conducted osteological analyses of identity, trauma, and time since death and the forensic pathologist conducted his/her own analysis. Three of the victims were discovered within structures, such as vehicles or boats. The submersion intervals ranged from a few days to approximately 30 years. As such, the decomposition observed on the remains varied but provided a taphonomic baseline for PMSI to be created.

Upon analysis, the data culled from the three types of variables (extrinsic environmental, extrinsic *corpus*, and intrinsic *corpus*) were consistent with expected patterns of decomposition. Skeletal inventories demonstrated an expected negative relationship between submersion interval and percentage of body recovered, with the exception of those remains contained within structures. Remains with the shortest postmortem submersion intervals exhibited soft tissue and organ retention, while those with the longest submersion intervals exhibited bone free from soft tissue. Remains displaying extensive adipocere, and thus delayed decomposition, encountered factors conducive to adipocere formation, including neutral or mildly alkaline pH levels and warm temperatures (between 15-30° C), around the time of initial submersion, which was consistent with previous research. Bone that was exposed to adipocere evidenced greatly reduced bone integrity through cortical flaking and trabecular bone wasting. Variables which appeared to greatly impact the rate of decomposition with relation to submersion interval included the following: water temperature, pH level, depth of immersion, presence/absence of clothing, and whether the remains were submerged within a structure (protected from flora, fauna, and water currents).

Therefore, when medical examiners, anthropologists, and law enforcement recover remains after long-term aquatic submersion, the creation of a subaquatic taphonomic baseline is essential to establishing an accurate postmortem submersion interval.

**Forensic Anthropology, Decomposition, Postmortem Submersion Interval**

# H94 Taphonomic Degradation to Bone Through Scavenging by Marine Mollusks of the Class Polyplacophora

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After attending this presentation, attendees will gain a greater understanding of an underreported type of marine scavenging, the damage caused to skeletal elements by marine mollusks, and the characterization of such with special regards to differentiation from other types of scavenging. Specifically, mollusk scavenging has been compared to rodent gnawing; attendees will learn characteristics differentiating these two distinct processes.

This presentation will impact the forensic community by increasing forensic knowledge on the taphonomy of long-term marine exposure, while contributing to the growing body of information regarding time elapsed time since deposition, and providing forensic practitioners with "real-life" examples of taphonomic processes which have previously only been discussed without adequate visual documentation.

Knowledge of taphonomic processes is essential to the forensic anthropologist in estimating elapsed time since death and postmortem influences on remains. Terrestrial taphonomy is well understood and the subject of numerous experimental and regional studies, and yet marine taphonomy in comparison is relatively unexplored. This is problematic for forensic professionals in coastal areas, where marine contexts play a significant role in the postmortem interval.

In February 2007, recreational divers off the coast of British Columbia, Canada recovered skeletal elements from a depth of approximately 20 meters / 60 feet below sea level. Suspecting them to be human, the divers turned over the skeletonized elements to the local police (RCMP), who contacted forensic anthropologists at the Centre for Forensic Research at Simon Fraser University for examination of the remains.

The skeletal elements were clearly of non-human origin. However, the unique taphonomic modification to the bone inspired further examination and consideration. Significant cortical bone mass was removed in deep, wandering channels and concavities. Several mollusks of the class *Polyplacophora* were adherent in the concavities at the time of recovery. Within the concavities, minute lines are etched in roughly parallel, though undulating, striations. These are likely the result of scavenging by the associated *Polyplacophora* who, like other mollusks, constantly expand and remodel their protective shells with calcium carbonate, requiring a high intake of nutritional minerals. *Polyplacophora* feed with a long, tooth lined radula used for scraping up sediments and substrates for their nutritional content. The microscopic teeth lining the radula are tipped with magnetite and must be constantly produced. As teeth are damaged and lost during feeding, new teeth are continuously mineralized and advanced into position. This constant need for dietary minerals suggests that submerged skeletal elements may be an ideal substrate for scavenging by various *Mollusca* species.

This case review highlights how long-term scavenging by marine mollusks, specifically of the class *Polyplacophora*, influences the taphonomic degradation of the skeletal element. Photographic and radiographic documentation illustrate the characteristic nature of damage to the bone and provide a visual example of a regionally unique taphonomy which, until now, has only been superficially discussed in forensic taphonomic literature.

**Marine Taphonomy, Scavenging, Forensic Anthropology**

# H95 Skeletal Remains in a Fluvial Environment: Microscopic Evidence of Glycoproteinous Adhesive of Balanus Improvisus on the Occlusal Surface of Mandibular Teeth

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After attending this presentation, attendees will understand how microscopic analysis can detect glycoproteinous adhesive of barnacles and may help determine placement of remains in a fluvial environment. This study may help determine the placement of mandibular or maxillary teeth, and possibly skeletal elements, in a fluvial environment even when only one or a few teeth are present and, with further research on the glycoproteinous adhesive, it may help determine postmortem interval in fluvial environments.

This presentation will impact the forensic community by showing the necessity of microscopic analysis of the human skeleton even if macroscopically it appears nothing is present on the remains. Microscopic analysis revealed this mandible had been in a fluvial environment even though there were no visual indicators it had been.

Barnacles are crustaceans that typically inhabit shallow salt waters with 75% living in water depths of less than 100 meters and 25% inhabiting intertidal zones. During the larval stage, the cypris antennae secretes a glycoproteinous adhesive that attaches to a hard substrate prior to metamorphosing to an adult form. Barnacles adhere themselves to substrates such as rocks, ship hulls, and oyster beds.

In a recent microscopic examination of human dentition from skeletal remains brought to the Galveston County Medical Examiner's Office, Texas City, Texas, the adhesive protein of *Balanus improvisus*, an acorn barnacle, was found on the occlusal surfaces of left $PM_1$, $PM_2$, and

$M_1$ of the mandible. Other than the mandible found separate from the remaining skeletal elements, no other fluvial indicators, such as algal staining, circumferential staining, silt staining, or matrix compaction were present on the mandible. Without the microscopic analysis of the dentition, the presence of the adhesive protein and ultimately the determination that this mandible had been in a fluvial setting may not have been discovered.

*B. improvisus* found on $PM_1$ measured 1.99mm in diameter. Two small adhesions of *B. improvisus* were found on $PM_2$, one measuring 4.09mm and the other measuring 1.82mm. The adhesion on $M_1$ measured 1.55mm.

The adult *B. improvisus* lays eggs which hatch into the larval stage. In the initial larval stage, the nauplius larva has a pelagic swimming period before it molts into a bivalve larva, known as a cypris. The cypris searches for a short period of time for a settlement spot and eventually settles on a substrate where it lives out its adult life. In general, most barnacles live two years. The larva of *B. improvisus* typically settles during the late summer or early fall months and grows into an average diameter size of 10mm but can reach diameters of 20mm. *B. improvisus* has been found in the estuarine system in Galveston Bay, Galveston, Texas.

Research shows that *B. improvisus* prefers smooth surfaces with which to attach. The highly cross-linked proteins deposited to attach to the substrate are so strong that they can remain on the substrate even after the carapace (body) of the barnacle is gone.

This case report will show how the detection of the glycoproteinous adhesive of the barnacle may: (1) help determine placement of remains in a fluvial environment sometime during the taphonomic process, (2) help determine placement of mandibular or maxillary teeth, and possibly skeletal elements, in a fluvial environment even when only one or a few teeth are present, and (3) with further research on the glycoproteinous adhesive, it may help determine postmortem interval in fluvial environments.

*Balanus Improvisus*, **Glycoproteinous Adhesive, Human Dentition**

## H96 Cremated Tooth Morphology: A User's Guide to Identification

*Elizabeth M. Danner, BA\*, School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, PR1 2HE, UNITED KINGDOM*

After attending this presentation, attendees will understand how to identify burnt and fragmented dentition using external crown and root as well as internal pulp cavity and root canal morphology.

This presentation will impact the forensic community by providing a detailed methodology for the identification of cremated dentition and by enabling practitioners to rapidly identify cremated dentition for the estimation of the minimum number of individuals, creation of postmortem dental records and interpretation of burn patterns.

Conventional dental identification focuses on crown morphology as the most unique and most easily observed dental trait, but is of little use in cremations where the crown enamel commonly shatters into tiny shards. Dentine roots survive cremation and may even survive pulverization in a modern crematorium, but few authors address the subject of how to identify cremated dental fragments.

To create a stringent identification system for forensic applications, this study applied population frequencies of both external and internal dental morphology to identify the dentition of the Late Bronze Age West Overton G 19 Cremation Cemetery housed at the University of Central Lancashire. An archaeological sample was chosen because the implantation of dental appliances alters tooth morphology and has been documented to impact cremation fragmentation patterns. Fragments

from a previous analysis were observed with the aid of a magnifier and macroscope.

Though a text book will picture the average tooth, actual teeth exhibit a great degree of variation in cusp, root and root canal number due to individual and regional differences. To account for this variation, identification was broken into six levels of certainty from broad traits shared by several teeth to very specific traits unique to a single tooth. The "uniqueness" of each trait was determined by White population frequencies reported in the dental literature and observations of British Medieval, Bronze Age and Victorian skeletons housed at the University of Central Lancashire.

Fragments were identified to 6 increasing levels of certainty: (1) **Position** as anterior or posterior was determined by the number of cusps, roots, and pulp horns and the curvature of the cementum enamel junction, (2) **Tooth type** of incisor, canine, premolar or molar was based on the number and shape of cusps, pulp horns, roots, and root canals, (3) Differences in morphology, size and thickness of dentine and enamel between permanent and deciduous teeth identified **dentition set**, (4) Jaw as maxillary or mandibular was determined by differences in root, cusp, pulp horn and root canal shape, (5) **Side** determination as left or right was only possible when the arrangement of cusps or roots identified the mesial or distal side, such as the placement of the hypoconulid distally in mandibular molars, and (6) Identification of tooth **number** as first, second or third was also difficult as many teeth vary little or inconsistently between sequential teeth.

The analysis of 479 dental fragments of 18 individuals (8 juveniles and 10 adults) identified the majority of fragments (74%) to position, most (66%) to dentition set and type, about half (45%) to jaw, and some to side (15%) and number (10%). Of the 479 fragments, only 26% did not contain enough features for identification, compared to the 50% not identified in a previous analysis using external morphology alone.

In forensic applications, this marked increase in identification may improve the estimation of the minimum number of individuals (MNI) and reconstruction of perimortem events. Anterior teeth have been observed to suffer a higher degree of fragmentation and burning in vehicular crashes, but survive intact when the body decomposes prior to incineration. The high identification rate (74%) of fragment position may then indicate the timing of cremation in relation to time of death. The identification of fragments to dentition and type (66%) is used in estimation of the MNI through repetition of teeth and presence of deciduous teeth.

**Dentition, Cremation, Identification**

## H97 Going Green: Environmentally Sound Practices in Human Decomposition Research and Laboratory Settings

*Michelle D. Hamilton, PhD\*, and Jerry Melbye, PhD, Texas State University-San Marcos, Department of Anthropology, 601 University Drive, ELA 273, San Marcos, TX 78666-4616*

After attending this presentation, attendees will have a better understanding of specific policies and procedures that can be implemented in their own practices to establish ecologically sensitive protocols for both forensic anthropological laboratory and research work in the handling, processing, and curation of human remains.

This presentation will impact the forensic community and the broader public at large by introducing ecologically-friendly approaches that researchers working with fresh and decomposing human remains can utilize in order to lessen the impact of harmful chemicals and products on the surrounding environment, and will identify areas where these changes can be made cost-effectively and with a minimum of interference or disturbance in already established practices.

The Forensic Anthropology Center at Texas State (FACTS) is now host to one of the largest outdoor human decomposition laboratories in the world. As a result of initial negative public reaction towards the establishment of this facility, the initially perceived negative concern (the introduction of decomposition, pollutants, and other harmful contaminants into the environment) was turned into a positive attribute. A "green" strategy has been consciously applied in as many aspects of the program as possible. The location of the Forensic Research Facility is just a few miles south of Austin, Texas, a city often ranked within the top ten of America's greenest communities in terms of recycling, energy, natural space, and transportation statistics.

In order to incorporate this same spirit of environmental responsibility, FACTS is making a concerted and systematic effort to incorporate various aspects of eco-friendly practice at multiple levels, including: (1) in addition to previously established avenues in the acquisition of body donations, the targeting and soliciting anatomical gifts from ecologically-minded citizens, (2) the acquisition of an alternative flex-fuel vehicle to use for both body donation retrieval and transportation to forensic anthropological crime scenes, (3) an adjustment of the procedures employed during the placement of human bodies for open-air, buried, covered, or surface decomposition scenarios to reflect the most environmentally safe options, (4) the products used in the cleaning and processing of human remains are largely composed of biobased, biodegradable enzymes, degreasers, and other naturally-derived products for body processing and skeletal preparation, and (5) the equipment and materials used in the archiving and curation of skeletal remains are specifically chosen to represent those items that are multiple-use, recyclable, or otherwise environmentally responsible options. FACTS is situating its anatomical body donation program into the bigger picture of ecological awareness by also promoting it to those individuals looking into "green burial" options, a newer trend in funeral services viewed as a natural alternative to traditional chemical embalming and coffin burials. In addition, in an effort to avoid the use of dangerous substances and to reduce the release of harmful chemicals into the soil and groundwater at the open-air decomposition facility, FACTS will not accept bodies into the body donation program that have been previously embalmed or preserved.

While FACTS has not achieved a completely green operation (and will never do so based on the realities of dealing with biohazardous waste, the need to observe universal precautions, and other intractable practicalities of forensic anthropological work), the laboratory is striving towards incorporating a full-range of alternatives and options that take advantage of newer products, technologies, and trends in the move towards a more ecologically-minded approach to research, community service, and laboratory protocol in forensic anthropological settings.

**Green Practice, Human Decomposition, Forensic Anthropology**

## H98 A Study of the Human Decomposition Sequence in Central Texas

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After attending this presentation, attendees will understand the decomposition sequence for human remains found in the context of this study in the late spring and early summer seasons of South Central Texas. Professionals regularly involved in investigations of unidentified remains will benefit from the decomposition information and visuals presented.

This presentation will impact the forensic community by establishing a preliminary decomposition baseline for human remains in Central Texas and regions of similar climate and geography. This study provides the forensic community with the foundation for a new and original data source tailored to specific environmental conditions and compared against previously published descriptive and quantitative studies.

Understanding the human decomposition sequence from varied geographic locations provides those charged with the investigation of unidentified human remains a tool for more accurately estimating the postmortem interval. Attendees of this presentation will gain insight into the timing and mechanisms of the decomposition sequence for human remains found within the context of this study during the late spring and early summer seasons of South Central Texas. Professionals regularly involved in time since death estimations of unidentified remains found in outdoor settings will benefit from the decomposition information, timelines, and visuals presented.

The outdoor decomposition of human remains involves a suite of complex, highly variable processes. Early processes including autolysis, putrefaction, and insect activity are dependent on environmental conditions, particularly temperature and humidity. Other decomposition processes, such as animal scavenging, are also site specific to the local environment and its faunal constituency. Due to such dependencies, a "one size fits all" decomposition model is unrealistic. It is imperative that ecologically distinct regions establish specific benchmarks by conducting controlled analyses that consider local conditions. Although retrospective and experimental human decomposition studies have established decay rates for specific eco-locations, such studies have been limited and primarily confined to the Southeastern and Southwestern United States. In Central Texas similar studies have been performed using pigs (*Sus scrofa*) and other nonhuman substitutes; however, no study to date has utilized intact human remains. This report provides summarized data from the first controlled field study involving human remains at the Forensic Anthropology Research Facility, Texas State University-San Marcos.

A donated human cadaver was placed at the open-air laboratory and regularly observed over a ten-week period. Data collected included the visual assessment of the stages of decomposition, insect specimens and observations of insect activity patterns, and weather conditions recorded at the permanent weather station located at the research facility. Additionally, the data were utilized to test a recently developed quantitative method for estimating the postmortem interval. Although two unexpected events occurred during the study, general results indicate a high degree of similarity with decomposition studies originating in the Southwestern United States, as well as preliminary support for a quantitative approach.

The purpose of this study was to establish a preliminary decomposition baseline for human remains in Central Texas and regions of similar climate and geography. This study provides the forensic community with the foundation for a new and original dataset—one tailored to these specific environmental conditions, and compared against previously published descriptive and quantitative studies. Further research is critical for the continued refinement of this initial study, and future studies should include observations in different seasons and varying depositional and burial contexts.

In addition to presenting the results of this study, the authors will briefly report on the current state of the Forensic Anthropology Research Facility, the largest and newest open-air decomposition laboratory in the world, and the third facility in existence explicitly utilizing donated human remains along with the University of Tennessee at Knoxville, and Western Carolina University. Expansion, construction, and current and future services will also be outlined, as will ongoing and future research designs which will build upon and complement this project to provide a better overall picture of the human decomposition sequence in Central Texas.

**Decomposition, Postmortem Interval, Accumulated Degree - Days**

## H99 Forensic Osteology Research Station (FOREST): The First Donation

*Cheryl A. Johnston, PhD\*, Western Carolina University, Department of Anthropology & Sociology, 101 McKee Building, Cullowhee, NC 28723*

After attending this presentation, attendees will come away with an enhanced view of human decomposition in the Blue Ridge Physiographic Province of North Carolina based on a case study of the first donation to be placed in the Forensic Osteology Research Station in Cullowhee, North Carolina.

The expected impact of this presentation on the forensic community is to begin to fine tune our understanding of human decomposition via research focused on patterns of decomposition and their environmental specificity. Instead of waiting until sufficient data are generated to report large scale patterns in more than one physiographic zone (which could take decades), this presentation will benefit the forensic community by reporting observations as they are made in hopes that each individual donor can inform our audience in some way. An additional impact of this work is that collaborations will be sought with researchers at other institutions with outdoor decomposition laboratories and those planning future decomposition laboratories.

The purpose of this study is to document the decomposition of the body of an adult male in the Blue Ridge physiographic province of North Carolina during the summer of 2008. On June 24, 2008, the clothed body was placed directly on the soil in the supine position on a south facing slope with the head inclined above the feet. The donor was placed in an area that is partially shaded by the forest canopy much of the day. Observations of decomposition and the environment were made and photographs were taken daily or every other day for the first month and weekly thereafter. Average temperatures during late June and July ranged from highs in the mid eighties to lows in the upper fifties and just over five inches of rain fell.

After 48 hours, fly eggs began to hatch and exposed areas of skin were in the beginning stages of sloughing. There was little odor until the fifth day and the odor never became strong. By the third day the skin had begun to darken and two days later the lips had turned black and parted. First and second instar fly larvae and adult beetles were observed on day five. Bloating was noted after one week and at this point tissue reduction of the head and neck was well under way. Third instar fly larvae were present in large numbers by day eight. Portions of the facial skeleton, left clavicle and left first rib were exposed by the ninth day. Migration of fly larvae began on the twelfth day and bloating began to recede. Numerous beetle larvae were present early in the third week and fly activity slowed. By week four the soft tissues of the skull and left upper thorax were greatly reduced but much soft tissue remained on the right shoulder, abdomen and appendages and arthropod activity was minimal. During weeks five and six the tissue of the arms and legs reduced, but no bone was exposed.

Prior to placing the donation and in order to collect terrestrial arthropods six pitfall traps were installed in the area where the body was to be placed, three pitfall traps were installed in other areas of the facility, and six pitfall traps were installed well outside the facility. The pitfall traps were collected at various intervals before and during decomposition. Prior to placing the donation as well as during decomposition, leaf litter samples for the collection of leaf litter fauna were collected in the facility and surrounding area. Leaf litter samples were processed in the laboratory using a Berlese funnel. Additional collections were made using sweep nets and forceps during decomposition. Arthropods observed include members of the families Calliphoridae, Silphidae, Staphylinidae, Histeridae, and Vespidae, among others.

**Decomposition, Environment, Arthropod**

## H100 Taphonomic Signatures of Animal Scavengers in Northern California

*Eric J. Bartelink, PhD\*, and Lisa N. Bright, BS, California State University-Chico, 400 West First Street, Department of Anthropology, Butte #311, Chico, CA 95929-0400*

After attending this presentation, attendees will gain a greater understanding of animal scavenging patterns on human remains from the Western U.S. The goals of this research are to: (1) document taphonomic signatures on human remains due to carnivore and rodent scavenging in northern California, and (2) address challenges in time-since-death estimates using taphonomic indicators.

This presentation will impact the forensic community by highlighting important considerations for assessing taphonomic signatures on human remains due to animal scavenging, as well as implications for time-since-death estimations.

Although taphonomic research involving animal scavenging has had a long history in paleontology and archaeology, only a handful of studies have focused on the scavenging of human remains from forensic contexts. Many of the forensic cases submitted to the Human Identification Laboratory at California State University, Chico (CSUC-HIL) derive from outdoor contexts and show extensive evidence of scavenging by carnivores and rodents. Northern California's extensive forests and rural landscape are home to a number of key scavengers, including black bears, coyotes, squirrels, opossums, raccoons, and rats. The especially high frequency of carnivore gnawing marks on bone indicates that bears and canids (coyotes and dogs) are among the most active scavengers of human remains in the area. However, rodents also play a significant role in the scavenging of human remains.

This study examines 21 forensic cases involving animal scavenging submitted to CSUC from 1986 to 2008. The majority of cases (n=16) are curated at the CSUC-HIL, with the remaining (n=5) examined through previous case reports. Each case was inventoried in detail, and all skeletal elements were examined for the presence of tooth impact marks. Elements that showed clear evidence of pits, punctures, and furrows were scored as carnivore gnawing damage. Similarly, linear, parallel striations were scored as evidence of rodent gnawing damage. To facilitate analysis, crania, ribs, hands, and feet are treated as single units rather than as separate elements. The frequency of each element represented was compared with the frequency affected by scavenging to evaluate the relationship between element survivorship and scavenging frequency. General patterns of involvement for carnivores and rodents are documented.

Because the recovery of remains was primarily conducted by law enforcement, the representation of elements may be more informative of the recognizability and size of skeletal elements than actual element survivorship. For example, 95.2% of crania, 88.1% of femora, and 83.3% of innominates were recovered, all which represent large elements easily recognizable as human. In contrast, small elements of the feet, hands, and the patella are the least represented (52.3%, 33.3%, and 28.6 %, respectively). When elements were ranked by their representation and compared by scavenging frequency, no correlation was found (rho = 0.162, p = 0.535).

Overall, 31.2% (n=205) of the 658 total elements show evidence of animal gnawing, with carnivore damage accounting for 27.5% and rodent gnawing for 3.6%. Of the elements with evidence of scavenging, the majority are associated with carnivore damage (carnivore = 88.3% vs. rodent = 11.7%). For carnivore scavenged remains, the highest prevalence is found for elements of the lower limb. Although smaller elements are more likely to be consumed during scavenging activity, all element types are well-represented in the dataset. Of particular note is that rodent scavenging is primarily found on the skull and large appendicular elements. For example, rodent gnawing was not observed on ribs, scapulae, clavicles, patellae, or the vertebral column.

Within-element patterns of carnivore scavenging are consistent with that reported in the literature—chewing activity is focused on nutrient-rich cancellous portions of proximal and distal segments of long bones, the pectoral girdle, and the pelvic girdle. For rodents, chewing behavior is guided by both the need to sharpen continuously growing incisors and also the need for minerals (e.g., calcium). Due to functional constraints of the rodent jaw, gnawing is often directed toward portions of elements that have sharp crests, ridges, or borders. The data indicate that rodent gnawing damage is mainly associated with regions of the skull such as the superior borders of the eye orbit, nuchal lines, mastoid processes, mandibular rami, and crests and muscle attachments of large appendicular elements.

The present study highlights the need to examine large samples of scavenged remains from different environments. The preliminary results of this study suggest that large carnivores (bears and canids) are the primary agents that modify human remains in outdoor contexts in northern California. Although rodents play a smaller role, nearly 12% of elements had significant damage due to rodent activity. Variation in the distribution of animal scavengers should be taken into account in time-since-death estimates.

**Taphonomy, Animal Scavenging, Time-Since-Death**

## H101   Raccoon (*Procyon Lotor*) Soft Tissue Modfication of Human Remains

*Jennifer A. Synstelien, MA\*, The University of Tennessee, The University of Tennessee, Department of Anthropology, 250 South Stadium Hall, Knoxville, TN 37996-0720*

After attending this presentation, attendees will be visually exposed to the unique scavenging strategies employed by the common raccoon; and the soft tissue manifestations thereof, over the course of soft tissue decay.

This presentation will impact the forensic community by explaining the theory that postmortem scavengers are said to be attracted to open wounds. Animal depredation can quickly destroy evidence of perimortem soft tissue injuries. While canid soft tissue modification of human remains is generally recognized, procyonid (e.g., raccoon) modification has not been described. Given the unusual dexterity of its forepaws, the soft tissue artifacts produced by the scavenging raccoon are unlike canid depredation patterns. Recognition of raccoon modification of human remains may be crucial for the interpretation of soft tissue injuries thereby assisting the medicolegal investigator in the assignation of the manner of death.

This paper characterizes the soft tissue artifacts produced by the scavenging common raccoon *(Procyon lotor)*, as photographically documented at the University of Tennessee's Anthropological Research Facility. From September 2003 through July 2004, multiple digital cameras were stationed at the Facility—a 2½ acre plot of land set aside for human decomposition research—to record the nocturnal behavior of small mammal scavengers. Post-July 2004, the cameras were sporadically operated through spring 2006. Near daily visits to the facility in daylight enabled detailed photographic, and written, documentation of soft tissue changes due to any previous night's activity. The accumulation of digital video, and photography, has produced an archive of imagery documenting the condition of the body at the time of placement and the location, and timing, of soft tissue modification.

Raccoons (*Procyon lotor*), in the order Carnivora, can be found throughout much of the United States. Although highly adaptable to diverse habitats, they prefer hardwood forests near streams, lakesides, or other bodies of water. They may establish dens in hollow trees, abandoned ground burrows, brush piles, caves or rock piles, drain pipes, and in, or under, buildings and structures. Urbanization has attracted many raccoons into metropolitan areas due to easily obtainable food,

water, and shelter. Exceptionally inquisitive, their unique dexterity enables them to manipulate objects and probe crevices extracting contents within reach for examination. The raccoon is highly omnivorous and forages at night for a variety of foods including fruit, berries, nuts, fish, mollusks, snails, earthworms, insects, crayfish, clams, frogs, turtles, carrion, and small rodents and birds as well as their eggs. By watching the behavior of other raccoons, they may incorporate new foods into their diet—such as corn, grain, vegetables, pet food, birdseed, and garbage. As an urban pest, they are known to uproot lawns while 'grubbing' for insects and their larvae. Melon growers recognize signature raccoon damage by the single hole cored through the rind with extraction of the interior's fleshy fruit.

The body donation program at the University of Tennessee, Knoxville provides for the unique opportunity to view nocturnal scavengers undeterred by chain link and privacy fencelines. As an excellent climber and an acceptable digger, raccoons have been frequent visitors to the Facility for several years. Human donors, and/or donor families, are aware that bodies decompose in a natural outdoor setting.

**Taphonomy, Postmortem Scavenging, Common Raccoon**

## H102   Estimating Ancestry Through Nonmetric Traits of the Skull:  A Test of Education and Experience

*Amber D. Wheat, BS\*, 232 Evan Liberal Arts, 601 University Drive, San Marcos, TX 78666*

After attending this presentation, attendees will understand the effects that education, experience, and the geographic region in which one works have on the accuracy of nonmetric ancestry determination. Statistical research results pertaining to the nonmetric method of determining ancestry will also be presented.

This presentation will impact the forensic community by making the forensic anthropological community aware of the effects that education and experience in forensic anthropology have on the accuracy of ancestry estimation using nonmetric traits.

The identifying characteristics of any unknown skeleton are age, sex, height, and ancestry. Among these four biological identifiers, ancestry is possibly the most difficult to assess. There are two main ways of estimating the ancestry of a skull: metric and nonmetric. The metric method involves twenty-one standard measurements that are entered into a computer based program (FORDISC 3.0). The program compares the measurements to those in a database containing measurements of known skulls from twenty eight populations. The program then classifies a particular skull into a population group. The nonmetric method involves a visual assessment of the skull, using the overall structure of the skull to classify it into an ancestral group. These two methods are often used together to determine the most precise ancestry of a skull.

Many of the methods used for ancestry determination are considered subjective, especially methods of nonmetric visual assessment. Therefore the nonmetric method should be tested not only for the precision of each trait (intra- and inter-observer error), but also for the accuracy of these commonly utilized nonmetric traits among forensic anthropologists.

This study used three blind tests presented to professional forensic anthropologists as well as students of forensic anthropology. These three tests were conducted in different geographic regions of the country. A total of twenty-seven people participated in the study. Of the twenty-seven participants, six were in the PhD level, nine in the Master's level, ten in the Bachelor's degree level and two were currently in their undergraduate year studying physical anthropology. A questionnaire presented to each participant was used to determine a variety of things such as level of experience in forensic anthropology, level of education,

familiarity with nonmetric traits, and estimated number of forensic anthropological cases on which the participant has worked. Eight complete skull casts of known ancestry and identity were used to obtain a broad number of responses. The skull casts consisted of four Asian skulls, two European skulls, and two African skulls. Each skull was placed next to a poster that listed nonmetric traits for the three main ancestral groups: European, African, and Asian. Each participant determined the ancestry of all eight skulls and classified each into one of the three ancestral categories. If the participant could be more specific in determining ancestry (narrowing the ancestry down from Asian to Native American), they did so. Also, each participant listed the nonmetric traits he/she used to determine the ancestry of each skull.

The results of this study indicate there is no correlation between education level and/or professional expertise in the accuracy of using nonmetric skull traits to estimate ancestry. Based upon regression analysis, there is no significant difference between the accuracy rates of professional forensic anthropologists with a high level of experience and those with a low level of experience in determining the ancestry of a skull. Despite the fact that there is no difference in accuracy rates, those with higher levels of education were able to narrow down the ancestry of a skull more than those with lower levels of education. The first group, comprised of individuals with a PhD, correctly identified 68% of Asians, 64% of Africans and 75% of Europeans. The second group, consisting of individuals with MA degrees, correctly identified 76% of Asians, 61% of Africans and 76% of Europeans. The third group, consisting of students with a BA degree and students pursuing a BA degree, correctly identified 71% of Asians, 58% of Africans and 83% of Europeans. Overall, participants were able to correctly identify 79% of the Europeans, 72% of the Asians and 60% of the African skulls. This study is ongoing and an increase in participant sample size will further refine these results.

**Ancestry Determination, Nonmetric Traits, Biological Profile**

## H103 A Statistical Assessment of Cranial and Mandibular Morphoscopic Traits Used in the Determination of Ancestry

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After attending this presentation, attendees will learn the importance of utilizing a combination of both cranial and mandibular morphoscopic traits for ancestry determination.

This presentation will impact the forensic community by demonstrating the advantages of using statistical modeling and multivariate statistics for the determination of ancestry.

The determination of ancestry from the human skeleton is one of the most difficult and least precise aspects of the biological profile. However, an assignment of ancestry is of utmost importance for limiting the number of antemortem records used to compare with a postmortem profile and establish a positive identification. Ancestry is determined using a combination of craniometric analysis and an anthroposcopic assessment of a suite of morphological characteristics (morphoscopic traits) of the skull. Traditionally, emphasis has been placed on the cranium as the most diagnostic area of the skull, however, researchers have recently also turned to the mandible as a valid indicator of ancestry. This study looks at morphoscopic traits from the cranium and the mandible to see if, when used in combination, these traits increase accuracies in ancestry prediction. A second goal was to provide insight into the distribution of these traits among groups, with a careful consideration of the clinal distribution of morphological characters. Finally, several multivariate classification statistics were used to explore

these distributions and select the methods and variables with the lowest classification errors.

A total of 11 cranial traits and 12 mandibular traits were examined for 94 individuals (European Americans, n=48; African Americans=46) from the Terry Anatomical Collection housed at the National Museum of Natural History, Smithsonian Institution. Data were collected following standard descriptions and illustrations of each trait. Several statistical methods were used to verify the applicability of these traits for ancestry determination. Ordinal regression was used to determine the effect, if any, of ancestry, sex, and the interaction between ancestry and sex on each trait. The ordinal regression analysis suggests that ancestry has a significant effect on 8 cranial traits and 5 mandibular traits. Following the ordinal regression analysis, quadratic discriminant function analysis, CAP, logistic regression, and $k$-nearest neighbor statistics were generated to determine the classification accuracies of the combined cranial and mandibular traits. Cross-validated, stepwise classification accuracies ranged between 73% and 91%, depending on the variables used and the selected method of analysis. Logistic regression had the highest classification rate using these variables. A stepwise logistic regression analysis selected 5 cranial traits (IOB, MT, NAW, NO, and PBD) and three mandibular traits (torus development, chin prominence, and chin shape) and misclassified only 8% of the total sample ($\rho$=0.805, df=18, p>0.001). This level of accuracy is higher than previous studies using only cranial or mandibular traits, suggesting that the combination of these two regions of the skull should be considered during ancestry determination.

The error rates generated using these methods greatly enhance our ability to predict ancestry from the skull. Added benefits of using statistical modeling to predict ancestry from the skull include the removal of subjectivity from the analysis (i.e., the experience-as-evidence process) and the proper selection and weighting of the variables most useful for ancestry prediction.

**Ancestry, Nonmetric Traits, Multivariate Statistics**

## H104 Morphological Variations of the Cervical Spine as Racial Indicators: A Validation and Observer Error Study Using the Terry Collection

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After attending this presentation, attendees will learn to recognize the degree of bifidity in the cervical vertebrae and its relative utility as a racial indicator in building a biological profile.

This presentation will impact the forensic community by providing a validation study of a previously developed but infrequently used tool in forensic racial classification.

Constructing a biological profile from skeletal remains relies upon metric and morphological examinations. The frequency of certain morphological features may help forensic anthropologists assess sex and ancestry. Many of the methods used to assess ancestry are based on observations and measurements of the skull. When such remains are lacking, forensic anthropologists must look to measurements and observations of the postcranial skeleton.

This study undertook the validation of a method developed by Duray, Morter, and Smith (1999) in which the spinous processes of the second through seventh cervical vertebrae (C2 through C7) were assessed for one of three classifications of bifidity: bifid, partially bifid, and nonbifid. Their study relied upon the Hammann-Todd skeletal

collection. Their results indicate that C2 and C7 showed, respectively, 91% bifid and 98% nonbifid spinous processes for both ancestry groups (black and white), so the utility of other cervical vertebrae were examined. C3 and C4 were shown to be the most useful in determining race, with 76% of the study subsample being correctly classified (80% for white and 72% for black).

For the current study, a sample of 591 randomly selected skeletons from the Terry Collection was analyzed for the degree of bifidity using the system developed by Duray et al. In a blind analysis the authors scored C2 through C7 for degree of bifidity as indicated above. Results indicated that C2 and C7 showed, respectively, 87% bifid and 88% nonbifid spinous processes for the combined (black and white) groups. Highly significant differences ($p = < 0.01$) were found between the ancestry groups at C3 through C6, whereas a significant difference ($p = < 0.01$) between males and females was found only at C5. As with the original study, C3 and C4 appear to be the best predictors of race.

To test the repeatability of the method, two untrained observers assisted in assessing a subsample of 28 sets of vertebrae after reading the study by Duray et al. Prior to making their own observations, each untrained observer was also shown a sample of cervical vertebrae from the Terry collection to demonstrate the range of variation. Overall, classification disagreements between untrained and trained observers were noted in approximately 23% of observations. The authors also conducted tests of intraobserver and interobserver error by scoring a subset of 28 sets of vertebrae. Intraobserver error for the trained observers occurred in 8% to 12% of observations. All of the intraobserver errors involved discrepancies in the degree of bifidity (partial versus bifid or partial versus nonbifid) rather than strictly presence/absence (bifid versus nonbifid). Interobserver error occurred in 11% of observations and included several instances of disagreement in presence/absence.

In sum, bifidity in the cervical spine appears to be a useful method for racial assessment of the postcranial skeleton.

**Race, Vertebrae, Ancestry**


# H105   Hispanic Affiliation:  Definitions, Assumptions, and Biological Reality

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The goal of this presentation is to present the forensic anthropological community with the inconsistencies in the usage of the term Hispanic and to review the current methods used when attempting to identify individuals considered Hispanic.

This presentation will impact the forensic science community by discussing the importance of consistency, use, and meaning of the term Hispanic within the forensic science community in general and the forensic anthropological community in particular. The learning objectives are to present the forensic anthropological community with the inconsistencies in the usage of the term Hispanic and to review the current methods used when attempting to identify individuals considered Hispanic.

Hispanic individuals make up the fastest growing population within the United States and are now the largest minority group therein. The term Hispanic is officially considered an ethnicity by the U.S. Census Bureau and the Office of Management and Budget directive No. 15. On the U.S. Census, an individual must specify a race of Black or White and then indicate whether or not they are Hispanic or non-Hispanic. Further, the term Hispanic is based on a linguistic classification of the Spanish language and encompasses many different countries separated by major geographic and cultural boundaries. Moreover, many individuals that would be considered Hispanic in the U.S., indigenous Mayans for example, are not Spanish-speaking peoples.

In spite of widespread utilization, no accurate or accepted use of the term Hispanic exists within the forensic anthropological community. This paper will review the use of the term Hispanic by the forensic anthropological community and will review the current methods used when attempting to recognize and identify Hispanic individuals. Based on an evaluation of the forensic anthropological literature, there is no agreement on the meaning or usage of the term Hispanic. In the *Journal of Forensic Sciences* and in the *American Academy of Forensic Sciences Proceedings*, the term Hispanic has been previously described by forensic anthropologists as a race, an ethnicity, an ancestry, a biological category, and as biologically meaningless. More specific terminology has been used to describe this biological heterogeneous group including Southwest Hispanic, Mexican-American, migrant workers, or border crossers, while still others use terms relating to national origin.

How can forensic anthropologists tell the difference between a Southwest Hispanic, Mexican-American, a migrant worker, or a national origin group considered to be Hispanic when working with unknown skeletal remains? Hefner et al. (2007) presented results from a blind study that suggest forensic anthropologists have a difficult time recognizing and ascribing a racial or ancestry category to an individual self-described as Hispanic. Thus, the problem may not only lie with inconsistent and confusing terminology, but also with methods of identification.

Sledzik et al. (2007) suggested that in the 21st century forensic anthropologists will abandon racial classifications in lieu of geographic origins or population groups. This goal is certainly realized in research articles working with known populations, which are described by their geographic or national origins. However, when faced with an unknown set of skeletal remains, using a geographic origin in lieu of a generic term seems unlikely, especially for individuals considered Hispanic simply because the data is not available. Until more data become available that describe the range of variability that is inherent within the group currently known as Hispanic, applying a geographic label to an unknown set of skeletal remains will continue to be difficult. Differentiating southwest Hispanics from southeast Hispanics is currently possible in some situations due to contextual inferences, although further national and geographic separation is currently problematic.

**Hispanic, Biological Affiliation, Biological Profile**


# H106   Morphoscopic Traits:  Mixed Ancestry, Hispanics, and Biological Variation

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The goal of this presentation is to highlight several multivariate statistical approaches that are useful for classifying these seemingly heterogeneous populations, which are often described as hybrid groups evincing cranial morphologies shared between multiple ancestries.

This presentation will impact the forensic community in general, and the forensic anthropological community in particular, by exploring the distribution of morphoscopic traits in groups with complex population histories.

The past decade has witnessed a dramatic increase in the number of research articles and presentations on cranial morphoscopic (nonmetric) traits within populations in the United States. Sadly, methodological and interpretative strategies applying these traits to predict ancestry in a forensic context remain largely unexplored. Previously, Ousley and Hefner presented multiple statistical methods appropriate for use with morphoscopic traits, yet the approach most often used by forensic anthropologists still relies almost exclusively on the experience of the observer rather than the distribution of these traits within populations.

Recent research in the *Journal of Forensic Sciences* and in the *American Academy of Forensic Sciences Proceedings* underscores the ubiquity of the experience-as-evidence approach without acknowledging any inherent shortcomings.

By exploring the range in variation of several commonly used morphoscopic traits using a large, worldwide sample (n=845) that includes individuals of self-identified mixed-ancestry, Hispanics, Africans, Europeans, and American Whites and Blacks, this presentation will demonstrate that the old assumptions of trait distribution, and the emphasis given to the experience of the observer, are not only typological, but also lead to unempirical and often incorrect classifications of mixed ancestry. The results of this study suggest that classifying an individual to "mixed" ancestry based on discordant trait values would only be tenable if all ancestral groups have been "mixed" for some time. If that is the case, then forensic anthropologists can correctly conclude that every decedent is of "mixed" ancestry, although this would negate the role of ancestry prediction in the biological profile. Thus, what are forensic anthropologists to do when confronted with Hispanics—a population often described as a hybrid group evincing morphologies shared between American Whites, Native Americans, and Africans—if these seemingly isolated populations also present discordant trait values? Several statistical methods that account for variation in trait frequencies have cross-validated classification accuracies nearing 87 percent. In a three-way analysis (i.e., Native Americans, American Whites, and Hispanics) using 12 variables with an overall correct classification of 87%, the Hispanic sample had a cross-validated correct classification rate of 90 percent. The benefit of a statistical approach is thus twofold. First, the importance placed on the subjective experience of the observer is reduced, an attractive attribute in light of the *Daubert* ruling. The second benefit of a statistical framework is the attachment of variable weights in the analysis, empirically supporting and strengthening classification accuracies using morphoscopic traits, while accounting for the true nature of biological variation.

Morphoscopic trait analysis remains an essential factor in the prediction of ancestry because of the emphasis and importance forensic anthropologists have historically placed on these slight variations in cranial form. However, when the actual distribution of these traits is understood, the discordance of multiple traits should come as no surprise and should not be treated as evidence of admixture or hybridity. On the contrary, discordance is evidence against the typological approach to ancestry prediction and represents the true nature of the distribution of morphoscopic traits among human groups.

**Morphoscopic Traits, Quantitative Methods, Ancestry**

# H107 Shifting Morphological Structure: Comparing Craniometric Morphology in Founding and Descendant Populations

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The goal of this presentation is to explore the applicability of using cross-population data for individual biological profiling.

This presentation will impact the forensic community by highlighting the importance of understanding biological variation in the determination of ancestry in unidentified remains.

The Americas experienced an influx of morphologic diversity with the arrival of African slaves in the 17th Century. Derived primarily from countries along Africa's west coast, millions of Africans were transported in slave ships across the Atlantic Ocean, to either the Caribbean islands or North and South America. At present, more than 38 million U.S. residents are of African descent (U.S. Census Bureau 2007). Moreover, autosomal DNA markers suggest this segment of the American population exhibits 22.3 +/- 15.9% European admixture (Wassel Fry et. al 2007). Despite several generations of intermarriage and interbreeding between African-American and European-American populations, however, African-Americans are often treated as morphologically analogous to their founding population (i.e., Africans) in forensic analyses, particularly those which are nonmetric in nature. The conflation of these two populations may very well obscure patterns of morphological variation unique to African-Americans and thus could have major medicolegal implications for the identification of unknown remains.

The present study addresses this issue by applying geometric morphometric methods to the question of craniometric affinity in founding and descendant populations. The populations under investigation include native African slaves who died in Cuba (n=15) from the Morton Collection; modern Cubans (n=21) from a cemetery collection housed at the Museo de Montane, Havana; and, modern African-Americans from the Terry collection (n=47). Nineteen three-dimensional type 1 and type 2 anatomical landmarks were collected. The landmark data were transformed by generalized Procrustes analysis (GPA) which optimally translates, scales, and rotates the points into a common coordinate system. Multivariate statistical analysis was then conducted on the newly derived shape variables. In order to reduce dimensionality, a principal component analysis (PCA) was performed on the covariance matrix of the aligned coordinates. A multivariate analysis of variance (MANOVA) test, performed on the first 14 principal component scores accounting for approximately 83% of the total variation, detected significant shape differences among the groups (F =14.38; df=28, 134; Pr >F= <0.0001). A discriminant function analysis was conducted using the principal components to allocate crania into groups using crossvalidation or n-1 method. The modern African-Americans were correctly classified 97.87% of the time, while the African slaves and modern Cubans were correctly classified 100% and 52.38% of the time, respectively. Almost half (47.62%) of the modern Cubans were misclassified as modern African-Americans. The higher misclassification rate of the modern Cuban sample most likely reflects the greater admixture (Spanish) proportion of the sample. In addition, the misclassification of the modern Cubans into the African-American instead of the African slave sample may reflect the greater proportion of admixture in the Terry sample and the more homogenous nature of the slave sample.

Based on these results, the descendant populations under investigation (i.e., modern African-Americans and Cubans) are distinct from their founding population. Moreover, generations of admixture have produced two populations which bear more similarity to one another, in terms of potential misclassification, than native Africans, despite both geographic and cultural distance. These findings highlight the fluidity of cranial morphology within descendant populations. Incorporating such information into standard forensic practice may allow for a more informative assessment of unidentified human remains than is possible under current classification schemas of race in the United States.

**Ancestry Determination, Populational Admixture, Geometric Morphometrics**

## H108   Non-Metric Trait Expressions Most Prevalent in Undocumented Border Crossers of Southwest Hispanic Descent From the Pima County Office of the Medical Examiner

*Carolyn V. Hurst, BA\*, 354 Baker Hall, East Lansing, MI 48824*

The goal of this presentation is to inform attendees about the prevalence of certain non-metric traits in Hispanic individuals found in the desert near Tucson, Arizona.

This presentation will impact the forensic community by presenting a suite of features that can help characterize individuals of Southwestern Hispanic ancestry.

One of the foremost goals of forensic anthropology is to obtain a positive identification for a set of remains. This process begins with the assessment of the biological profile, including sex, age, stature, and ancestry, that can be used to delimit the list of missing persons that may potentially match the John or Jane Doe. While methods for determining sex, age, and stature have been standardized and accepted by the scientific community, ancestry remains a subject of contention. Much of this controversy is related to the stigma surrounding the subject of race and the reality that biological races do not exist. Although humanity varies along a continuum, ancestry is a socially assigned category that is based on an individual's physical appearance. While forensic anthropologists recognized this fact, they also realize the utility of such assessments as descriptive aides in the forensic context.

The issue of ancestry is further complicated by admixture that increasingly blurs the already arbitrary lines separating groups. One particular group that is characterized by admixture is Hispanics whose gene pool consists of variable influences from American Indian, Caucasian, and African ancestries. With this group rapidly growing and becoming the largest minority in the United States, it is important that forensic anthropologists can accurately classify these individuals. In 2008, Birkby *et al.* published a paper describing the nonmetric skeletal traits that were utilized at the Pima County Office of the Medical Examiner (PCOME) to identify Southwest Hispanic ancestry in the biological profile of undocumented border-crossers (UBCs). These traits include: shoveling of the anterior teeth, anterior malar projection, a short occipital shelf, less elaborate nasal sill, partial or no oval window visualization, molar enamel extensions, nasal overgrowth, a wide zygomatic frontal process, and femoral platymeria in the subtrochanteric region.

The goal of this investigation is to evaluate the prevalence of the traits proposed by Birkby *et al.* in identifying Southwest Hispanics. In addition, other nonmetric traits were also scored to assess how commonly they occur in this group. Overall, 28 nonmetric traits were scored on the remains of 65 suspected UBCs from the PCOME. These traits included nine traits from Birkby *et al.* (2008), 12 traits from Hefner (2007), and several others from multiple sources. The frequency of these traits was then evaluated in order to ascertain their prevalence in populations of Southwest Hispanic descent.

Results indicate that all of the trait expressions described by Birkby *et al.*, except for nasal overgrowth, occurred in higher frequencies in the sample than alternative manifestations of those features. In addition, it was found that other traits may also be characteristic of those of Southwest Hispanic ancestry, like the presence of Wormian bones, venous markings, moderate interorbital breadth, lack of post-bregmatic depression, and moderate posterior zygomatic tubercle.

Establishing a suite of features that can be used to identify individuals of Southwest Hispanic ancestry is an important endeavor to aid in the identification of a growing group of people in the United States. Despite the admixed nature of this group, certain trait expressions hold potential to accurately assess their ancestry.

**Nonmetric Traits, Ancestry, Southwest Hispanics**

## H109   Skeletal Fracture Patterns in Documented Cases of Torture, Assault, Abuse, and Accidents

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The goal of this presentation is to provide a comprehensive analysis of skeletal fracture patterns and associated soft tissue lesions resulting from inflicted and accidental injuries. The patterns of injuries based on a variety of mechanisms are described to aid in a differential diagnosis of the cause and manner of death when confronted only with skeletal or decomposing remains.

This presentation will impact the community by reviewing more than 200 cases of blunt force trauma from a variety of mechanisms including torture, assault, and accidents to demonstrate intrinsic and extrinsic patterns that lead to a differential diagnosis of cause and manner of death. The outcome is a detailed analysis of the specific distribution and types of injuries resulting from a variety of blunt force mechanisms. This information is particularly critical in cases of enforced disappearances, extra-judicial execution, and torture as investigations into such cases often lead to multiple autopsies and varying opinions. The context of these types of investigations is further discussed.

Medico-legal death investigations into enforced disappearances and extra-judicial executions provide critical physical evidence that corroborates witness and survivor testimony about the crimes committed. One of the most ubiquitous forms of human rights violations globally is torture and is often associated with extra-judicial executions. In these types of investigations it is common that multiple autopsies may be preformed at the request of various stakeholders such as law enforcement, prosecutors, and families. Multiple autopsies often lead to conflicting opinions about the cause and manner of death, further complicating the investigation.

A 2005 investigation into a possible extra-judicial execution from Lagos, Nigeria resulted in three autopsies and the disappearance of critical skeletal evidence. It is an example of multiple opinions on the cause and manner of death ranging from 'suicide by jumping from a window' to a 'gunshot injury to the head with staging of the body to appear as though the victim fell from a window.' Police torture and deaths in custody in Nigeria have been documented by Human Rights Watch, Amnesty International, and even the government of the Federal Republic of Nigeria. The types of torture or cruel, inhumane, and degrading treatment of the victims most often includes repeated and severe beatings with rods, sticks, and other implements and may be associated with fatal gunshot injuries. The critical need for pathologists and anthropologists to be able to differentiate mechanisms of death from skeletal fracture patterns is exemplified by this case and discussed in greater detail.

The purpose of this paper is to investigate specific fracture patterns that resulted from various forms of blunt force trauma to identify patterns of injuries that will aid in the diagnosis of abuse, assault, or torture and help differentiate inflicted from accidental trauma as in the Nigerian example. Data for 200 cases are presented. The ages at death range from infants to 89 years old and include data from several sources. Skeletal fracture data comes from autopsy records dating 1987-2008 from the Lancaster County Coroner's Office in Lincoln, Nebraska; the Cook County Medical Examiner's Office in Chicago, Illinois; and the Knox County Medical Examiner's Office in Knoxville, Tennessee.

Documented cases of torture come from medico-legal investigations in Lagos, Nigeria and published case studies from a variety of scientific sources. In all of these cases, the mechanism of death was attributed to blunt force trauma (BFT) or gunfire injuries associated with BFT at the time of death.

The number and distribution of soft tissue injuries and skeletal fractures are documented for each mechanism of injury; documented torture (n=52), assault (n=8), child abuse (n=5), falls (n=12), crushing injuries (n=5), motor vehicle accidents (MVA, n=114), blasting injury (n=2), and small aircraft accidents (n=6). Further, the specific aspects and regions of bone fractures are summarized for each category. Distinct patterns of injuries for each of the listed mechanisms and best practice recommendations for differential diagnoses are provided. Further, the association between soft tissue lesions and skeletal fractures among various mechanisms of injury are discussed.

**Trauma, Skeletal Fractures, Torture**

# H110 Assessing Directionality of Low Velocity Gunshot Wounds to the Vertebrae: A Preliminary Study

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After attending this presentation, attendees will understand the importance of experimental research to determining the bullet trajectory in the vertebrae as well as main components that can be employed to determine the direction of fire: beveling, fragmentation, and fracturing. Incorporating all three factors is the most efficient method for determining direction of fire.

This presentation will impact the forensic community by introducing a method for determining direction of fire developed from experimental research as opposed to case studies. This will, in turn, give forensic practitioners additional ways to reconstruct the events of a crime, confirm or contradict a witness statement, and differentiate between homicide and suicide.

Firearms, handguns in particular, are common weapons used in violent crime in the United States and around the world. Penetrating trauma is the second leading cause of spinal injuries, and of the two major types of penetrating trauma to the spine (gunshot and stab wounds), the majority are gunshots. This prevalence amplifies the importance of stringent scientific investigations examining bullet trajectory in the vertebrae.

Publications on bullet trajectory in postcranial bones tend to be case studies rather than controlled experiments. Therefore, an experiment was designed to produce gunshot wounds from known directions in the vertebrae of domestic pigs (*Sus scrofa*). The hypothesis was that the determination of bullet trajectory in vertebrae shot with a low velocity weapon is possible using a method modified from that developed for the cranium.

Six vertebral columns from recently deceased pigs were shot with a 9mm full metal jacket bullet, three each a minimum of three times from the anterior and the posterior directions: once in the cervical, once in the thoracic, and once in the lumbar vertebrae. Another two vertebral columns were shot from directions unknown to the researcher in order to permit a blind study of the method developed from the wounds of known directions. A total of 23 gunshots of known direction to various sections of vertebrae made up the known sample.

Zones were assigned to each type of vertebrae (cervical, thoracic, and lumbar) based on the developmental anatomy of the pig vertebrae (Figure 1). In each zone, the researcher recorded the following categories of trauma: undamaged, fracture lines, comminuted, trabeculae exposed, obliterated, and unconnected piece.



**Figure 1**: a) Zone assigned to the anterior side of the cervical vertebrae, b) Zones assigned to the posterior side of the cervical vertebrae

The results indicated several general trends: (1) vertebrae shot from posterior-anterior were more fragmented and evinced more fracture lines on the neural arches and spinous processes, (2) the lumbar vertebrae were the least damaged overall, having the least number of adjacent vertebrae affected from each shot, and (3) although more cervical vertebrae were affected by each gunshot wound, the thoracic vertebrae displayed the most damage overall.

It was concluded that a method incorporating all of the factors (beveling, fragmentation, and fracturing) was effective for determining direction of fire. A blind study was conducted consisting of three trials: (1) determining direction of fire using only previous knowledge, (2) using examples from the known sample to assist, and (3) using the criteria developed by the researcher along with the examples to determine direction of fire.

The results of the blind study (Table 1) indicated a correlation between experience with gunshot trauma and the percent of cases the participants would successfully determine in Round 3. It also illustrated the complexity of determining trajectory on vertebrae and highlighted the potential benefits of future research with a larger sample size and human vertebrae.

**Table 1:** Summary table of the results from the blind study. Participants with "No Experience" had Master's or Doctoral level course work without any experience with gunshot wound cases. Participants who were "Experienced" had practical experience with gunshot wound cases

| Amount of Experience | Participant | Round 1 | Round 2 | Round 3 |
|---|---|---|---|---|
| No Experience | 1 | 3/6 | 4/6 | 3/6 |
| No Experience | 2 | 3/6 | 4/6 | 5/6 |
| No Experience | 3 | 2/6 | 4/6 | 4/6 |
| No Experience | 4 | 2/6 | 5/6 | 1/6 |
| No Experience | 5 | 3/6 | 4/6 | 4/6 |
| Experienced | 6 | 3/6 | 5/6 | 6/6 |
| Experienced | 7 | N/A | N/A | 5/6 |

**Gunshot, Postcranial, Trajectory**

# H111 A Radiographic Assessment of Pediatric Fracture Healing and Time Since Injury

*Christina A. Malone, BHS, BA\*, Michigan State University, Forensic Anthropology Lab, A-439 East Fee Hall, East Lansing, MI 48824*

After attending this presentation, attendees will gain an understanding of the bone repair process, radiographic manifestation of fracture healing in children, and the time schedule this healing process requires.

This presentation will impact the forensic community by demonstrating a schedule for fracture healing in children. Through the results presented, the schedule may enable forensic anthropologists to supply information on the timing of injuries to skeletonized remains that exhibit varying degrees of healing.

Although the physiological process of fracture healing has been well studied, there is little information available on the radiographic assessment of the rates of pediatric fracture healing. As children are still

in the formative phase of bone growth, healing of bone may occur at a faster rate than seen in adults. The goal of this study is to determine the applicability of radiographic assessment to pediatric cases involving fractures, produce stages of radiographic healing and descriptions, and provide a timeline for the stages of healing in infants and young children. It is expected to observe variation in the timing of healing based on the age of the individual and the bone injured.

This study aims to develop a series of stages to describe and measure the typical bone fracture repair process and to evaluate, for each subject, the timing of the repair of each fracture. This study examines a collection of radiographs (n=345) of lower limb and forearm bone fractures from 116 individuals between the ages of 0 and 5. A series of stages is developed to describe and measure the typical bone repair process for these individuals. The sample is segmented into age groups (0-1 years, 2-3 years, and 4-5 years), and the variation in fracture healing rates is examined among these groups. Within each age group, the variation in fracture healing between the lower leg and forearm is determined. ANVOA is performed on the mean number of days that it took for healing to attain specific stages in each of the groups.

The results of this project present a schedule of pediatric fracture healing, both through written descriptions of expected patterns in the healing process and in radiographic images of such stages. These images and descriptions will prove useful to forensic anthropologists when assessing radiographs or when assessing skeletal remains (in which case a radiograph could be taken to compare stages) and attempting to determine an estimation for when injuries may have occurred. The images and descriptions of the skeletal sample will also serve as an atlas for the fracture healing process. Finally, the study will determine the significance of the effect of age and skeletal location in fracture healing in infants and young children.

In conclusion, this study examines the utility of radiographs when examining traumatized sub-adult remains. Through radiological data on the healing rate of pediatric fractures, the forensic anthropologist will be given another tool to assess the timing of traumatic injuries in the assessment of skeletal fractures. Additionally, the presented study would be able to assist in confirming or refuting the proposed timing of injuries in pediatric cases.

**Radiographs, Children, Fractures**

## H112    Supra-Inion Depressions in a Pediatric Medical Examiner Sample: Support for a Synergy of Developmental and Biomechanical Etiologies

*Sharon M. Derrick, PhD\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

The goal of this presentation is to demonstrate the forensic significance of a small cranial anomaly, the supra-inion depression (SI), which has previously been associated with child death in Native American populations of archaeological age. Attendees will be provided with a description of SI morphology, a brief overview of the published literature regarding the anomaly, and the results of a review of crania from 659 medico-legal cases dating from 2005 - 2008 in which the maximum age of the decedent is 6 years. Case studies of crania that contain a supra-inion depression will be discussed.

The impact to the forensic community lies in the dissemination of information regarding this little understood skeletal anomaly, the circumstances in which it develops in children presenting to medical examiner/coroner offices, and the importance of the SI as a symptom of morbidity on autopsy.

Supra-inion depressions, concave features located in or lateral to the sagittal plane of the occipital just superior to inion, have been described occasionally in the bioarchaeological literature. However, the etiology and incidence of these anomalies in the medical examiner setting has not been adequately studied. The discovery of a non-infectious, classically shaped SI in a 17 month old child with TORCH syndrome during postmortem examination at the Harris County Medical Examiner's Office (HCME) in 2008 triggered a photographic review of all previously examined HCME medico-legal child death cases from the first six months of the year 2008 (112, 74 supra-inion regions clearly viewable). Due to the retrospective use of photographs, only the presence/absence of SI, associated infectious bone, sutural fusion, and distortion of the occipital, parietals and frontal were scored. Cause of death was recorded for all cases. Two additional cases from this time period with SI's were identified (3/74 = 4%), a two year old female with a history of cerebral palsy and epileptic seizures whose cause of death is bacterial sepsis, and a two year old male who experienced a delayed hospital death with fever, cause of death pending. The cranium of the female child has a flattened occipital and the vault is unusually tall. No infectious bone reaction is visible in the area of the SI. The cranial shape of the male who spent a week in a hospital bed prior to death is normal in appearance, but the bony margins of the SI appear inflamed. In light of these preliminary results, a retrospective review of 447 cases from the years 2005-2007, as well as physical examination of current cases, are ongoing.

The growth and development of the cranium is affected by a number of genetic/congenital disorders, for example, the various TORCH syndromes, Apert's syndrome, Crouzon's syndrome, and Pfeiffer syndrome. These disorders routinely result in premature synostosis of one or more of the major cranial sutures, causing the cranial bones to compensate in shape. Growth and development of the occipital is a complex process that derives from the fusion of four individual components: the squamous, two lateral components, and a basal component. Although it varies among individuals, fusion of the four components typically begins in the perinatal period and is complete by age six. Premature fusion of any of the major sutures during these ages can disrupt development of the occipital in relation to the four components and the other bones of the skull. The environment experienced by the child from infancy to six years can also impact the final shape of the occipital. Positioning of the infant on the back for long periods of time, especially if exacerbated by lack of movement resulting from developmental delays, can result in plagiocephalic or scaphocephalic abnormalities. Further, the supra-inion area is posteriorly projecting and the skin of the scalp is thin. The opportunity for bone involvement in this area following a skin infection may be increased. Bacterial and fungal infections, or even seborrheic dermatitis (cradle cap), may become severe in the absence of treatment, perhaps explaining the noted association between SI's and infection.

The association between the presence of SI's, syndromic abnormalities, developmental pressure from biomechanical forces, and presence of infection in medical examiner cases suggests that SI's may be an indicator of morbidity. These anomalies should be routinely observed and documented photographically during the pediatric postmortem examination.

**Supra - Inion Depressions, Occipital Anomaly, Forensic Anthropology**

## H113    The Recovery of Human Remains From a Fatal Fire Setting Using Archeological Methodology

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After attending this presentation, attendees will understand the value of applying archaeological recovery methods at fatal fire scenes

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not only to maximize the amount of human remains recovered but also the associated artifacts surrounding the death.

This presentation will impact the forensic community by providing insight into the utilization of essential skills for the recovery of critical evidence and a greater quantity of human remains.

There is a natural tendency for those involved in fire settings to become overwhelmed simply by the magnitude and destruction of the scene itself. One can easily become overpowered at fires where there is large loss, and the path the investigator must take may be obscured by the scale of the scene. Fire investigations are often complex and difficult to interpret at first blush. Because of the potential for the fire investigator to become fixated or pre-occupied, one must develop an analytical and systematic approach to scene investigation.

With strong emphasis being placed on the systematic approach to fire investigations, it is expected that fire investigators with experience and training in archaeological methods will successfully meet the rigorous test of the scientific method. A scientist observes the real world and draws conclusions from these observations. The observations are then tested to determine their validity. How then could archaeology or the application of archaeological methodologies assist the excavator at fatal fire scenes? "Archeology concerns itself with learning the details of everyday life as well as significant or unique events, arranging these reconstructions in chronological sequences to create histories, attempting to understand or explain why things happened the way they did…" (R.M. Stewart, 2002:1).

Keeping this in mind, refined techniques of human remains recovery, the location of associated artifacts, the observation of body positioning within the context of the structure, and scene analysis will allow for a more accurate analysis to move toward proving this hypothesis. The primary objective of the study profiled in this presentation is to employ and contrast the methods of recovery of human remains in a fire setting in an attempt to increase the contextual and associational data acquired for accurate event reconstruction.

This study basically involves a three-fold method; involving the use of "comparative" fires, the application of a questionnaire to over five hundred historical fatal fires within the Province of Ontario and firsthand fire excavations conducted in the everyday course of employment by the author.

The "comparative" fires involved existing standing structures, pre-staged with pig cadavers and artifacts associated with homicide, which were allowed to totally burn. Personnel who lacked formal training in the disciplines of archeology and anthropology conducted the initial search for human remains. Any recovered remains and artifacts were photographed, mapped *in situ* and collected. A second search team consisting of individuals experienced and trained in archeological techniques and a solid background in human osteology were utilized. A proper archaeological-style grid search was undertaken with any artifacts and human remains photographed, mapped and recorded.

The quantitative relationship between the items recovered by the two teams was profiled and documented. At this point, there have been four "comparative" fires conducted.

The historical portion of this study involves the application of an extensive questionnaire to over five hundred historical fires. The purpose of this questionnaire was to capture existing data involving the methods employed by previous fire investigators at these types of scenes by way of scene comparison and rate of recovery. The "day-to-day" scene data relates to firsthand knowledge of fatal fire scenes excavated by the author. The data obtained, including the amount of human remains recovered and artifacts associated to the fatal fire scene, are profiled in this method.

To date, the resulting recovery analysis has proven overwhelmingly that the application of archaeological methods at these types of scenes both supports and authenticates the utilization of these methods. Two case studies will be presented in the recovery of fatal fire victims and articles associated to the deaths, both within a structure and a vehicle.

**Fire, Fatal, Archeology**

## H114 From Scene to Seen: Post-Fire Taphonomic Changes Between the In Situ Context and the Medicolegal Examination of Burned Bodies

*Elayne J. Pope, PhD\*, University of West Florida, Anthropology Department, 11000 University Parkway, Building 13, Pensacola, FL 72701*

After attending this presentation, attendees will understand how the body's physical appearance changes from the *in situ* condition at the scene until its evaluation by the medicolegal examiner due to recovery, handling (fragmentation), and transportation.

This presentation will impact the forensic community by informing investigators about the physical changes that occur to fragile, burned human remains from the time of discovery, to recovery, to transportation, and to the medicolegal investigation (independent of the scene) of the physical condition of the burned human remains.

It is often taken for granted that the physical condition of the body examined at autopsy accurately represents how it first appeared at the scene – unaltered. This presentation shows how the body literally changes its physical appearance from the *in situ* condition of the untouched "scene" and demonstrates how its appearance becomes altered during recovery, handling (fragmentation), and transportation to the point when the body is later "seen" and evaluated by the medicolegal examiner.

Heat exposure transforms soft tissues and bone into charred, brittle, and fragmentary structures that break away from parts of the body during and especially after the fire. Experimental observation of human cadavers in fires shows that these fragile structures experienced further fragmentation and alteration of the body's appearance during normal field search and recovery of burned human remains. The processes of burning and the different variables of fire suppression, discovery, clearing/extraction, recovery, and transportation were observed and documented for 5 bodies in vehicles and 2 bodies in burn cells (furnished model rooms).

**Fire Suppression:** The different methods of fire suppression are: standard water jet stream, fogging, and natural extinguishment directly influenced the condition and appearance of the body after the fire. If the body is hit directly with a pressurized water jet stream from a fireman's hose, the fragments of soft tissue and bone become displaced around the body. Of equal importance is the displacement of debris that falls on top of and around the body, and collapse of supporting structures (furniture, flooring) where the body is positioned. Pressurized jet stream suppression caused greater fragmentation of the body and the surrounding environment, thus expanding the search area and disintegration of smaller elements from water saturation and drainage. The technique of fogging sends the same pressurized water through an aspirating nozzle, thus creating a shower effect of smaller water droplets and is less destructive to tissues of the body and the surrounding structural materials. Foam or detergent can be added, but produces a similar effect by minimizing the damage to the victim. However, the use of water suppression can have the potential to wash away trace evidence and alter delicate skeletal trauma and should be considered during the postmortem examination. Natural or self-extinguishment is where the fire dies out from lack of fuel, but keep in mind that the body is a fuel load and the body's fat can sustain a localized fire for hours, thus increasing the heat-related damage to the body.

**Search/Discovery:** Fire scenes are challenging since most materials are visually altered and camouflaged among the ash and debris, including burned human remains. For this reason, it may not be obvious that there is a victim present at the scene, particularly if they are buried under similar-looking debris. During the search, fragile remains may be walked over, crushed, and further fragmented before being discovered, or disturbed by initial removal of large furniture/objects and raking of

smoldering debris.  Post-fire breaks are easily identified by the crisp and bright colors of the margins, as opposed to heat-related and traumatic fractures with more uniform coloration with adjoining cortical surfaces.

**Clearing and Extraction:**  Before the body is even touched, the methods of access can cause further fragmentation.  For vehicles, different techniques of opening the doors, trunk, and roof will cause debris to fall on the body and movement of fragile remains.  Different power tools such as a reciprocating or circular saw causes vibration of the vehicle and body.  Hydraulic slow cutting saws (jaws of life) cause less vibration and movement, but still jar the body.  Access to the body requires that the door and trunk locks must be forcefully pried open, thus shifting the body's position.  For structural fire scenes, most of the larger construction debris (roofing, ceiling, walls, etc) must be removed to gain access to the remains and likewise causes fragmentation of brittle burned human remains.

**Recovery:**  The field recovery team may or may not have osteological training, thus the recovery may involve the selection of larger and identifiable parts, leaving smaller fragments and pieces of the victim at the scene.  Even careful handling of charred and calcined bone can result in fragmentation, especially when the larger parts are lifted and moved from the *in situ* context onto a sheet or body bag.  Dry screening (if possible) is the best way to collect smaller fragments of bone, thus insuring all of the evidence and parts of the victim are taken for postmortem examination.

**Transportation:**  Movement and placement of fragile burned remains into a body bag is guaranteed to cause fragmentation, especially if the body bag is not supported by a backboard or a rigid structure.  Picking up the flexible body bag at 2 or 4 points and movement from the scene means that the body's weight can crush loose fragments and causes more fragmentation from handling as the remains are moved from the scene, loaded and removed from a transport vehicle, transferred to a secondary gurney or surface, and then opened for the postmortem examination.  The thickness and rigidity of the plastic/fabric also should be considered as a contributing factor in pressing against the fragile remains, thus increasing fragmentation.

Often the bodies of fatal fire victims are examined independent of the fire scene.  The investigator should be aware that the condition of fragile, burned human remains changes its visual appearance from the point of discovery to the postmortem examination (independent of the scene) when the body's physical condition is analyzed as evidence for the medicolegal examination of the victim's manner and cause of death.  Examples will show the progressive stages and causes of fragmentation from the point of discovery to laboratory examination.

**Fatal Fire, Burned Human Remains, Cremation**

## H115  Human Cremains From a Controlled Car Fire

*Peer H. Moore-Jansen, PhD, Department of Anthropology, Wichita State University, 114 Neff Hall, Wichita, KS 67260-0052; Elayne J. Pope, PhD, University of West Florida, Anthropology Department, 11000 University Parkway, Building 13, Pensacola, FL 72701; and Laura B. Bennett, BS*, 1013 Wisteria Drive, Derby, KS 67037*

After attending this presentation, attendees will have learned what happens to a human body when it has been subjected to fire.

This presentation will impact the forensic community by presenting controlled research experiments in an area where very little research is available.

Within the past two decades, a wealth of taphonomic research has emerged that focuses on isolating and identifying a variety of controlled environmental variables in order to establish patterns of postmortem changes.  Vehicle fires are unique environments loaded with combustible materials of plastics, foam, upholstery, carpet, rubber, and petrol-based products, all housed within the small space of a metal frame.

The location and positioning of the victim's body within a vehicular fire was found to directly influence the extent and types of burn patterns.  Ten human cadavers and ten pigs were placed in different sections of cars: front seat, back seat, and trunk to:  (1) observe the differences in the extent and patterns of heat-related damage, and (2) the average length of time that the bodies continued to burn after the manufactured fuels (interior) had naturally extinguished.  Of course, each vehicle is unique in the amount and types of synthetic materials, compartment size (truck cab vs. minivan), interior construction (bucket or bench seat), age of the car's materials (older or newer), and the amount of ventilation during the growth stage of the fire (windows open or closed).  The salvage vehicles used in these burn experiments were manufactured before the year 2000.  Newer models have different synthetic plastics and materials, which would produce accelerated results.  On average, the vehicles burned for 45 minutes to an hour, while the bodies continued to burn for different lengths of time depending on their placement within the vehicle.

**Front Seat:**  Bodies positioned in the front seats, typically bucket seats with upholstery over a wire frame seat, initially provided protection to the back and legs.  As the fabrics and plastics burned away, the body remained supported on the wire frame seat frame, thus allowing ample circulation of heat and fire to all surfaces and more evenly distributed burn patterns of the body.  Movement of the body was observed as supporting combustible materials burned away.  For example, a body slumped over on the dashboard gradually lost this point of support and fell over into portions of the floor or driver's seat.  In some cases, the seat fell back, thus positioning the body into a prone position and partially into the back seat.  Since the body burned on a wire frame seat, it remained elevated in the fire environment and continued to burn for several hours (2+) after the car fire had self-extinguished.  The small fire burning under the body was due to the supply of melted body fat under the body and around the areas of the torso which continued to render the body into charred tissues and bone.

**Back Seat:**  Bodies positioned in the back seats had the least amount of heat-related damage when compared to those placed in the front seats and trunk of the same vehicle.  The back seats consist of minimal upholstery over a broad, flat metal bench, which burns away early during the fire.  Then the body remained in direct contact with the metal bench, thus preventing heat and circulation to points of contact, which resulted in partial burn patterns of only the areas exposed to the fire.  Likewise, there was not enough fuel to sustain burning of the bodies, nor ample materials to sustain the wick effect from the body's melted fat.

**Trunk:**  Bodies in the trunk, due to their protected environment, took longer for the fire to reach but burned intensely once the trunk was involved.  This required that the back seat upholstery burned away and exposed the perforated metal structure, thus allowing ventilation and direct heat to reach the body.  Some trunks housed a spare wheel, or at least a depressed wheel well. The presence or absence of a tire in the trunk was influential not only as a solid fuel source, but the metal rim elevated portions of the body and allowed the body's fat to pool there as a sustaining fuel source.  The trunk space became a miniature crematorium environment as air and heat circulated through the back seat and the burned out openings of the taillights.  Bodies in the trunk burned intensely for over 4 hours past the initial car fire and left most of the body as charred and calcined bone, with the exception of some adherent tissues of the bulky torso.  Their condition and preservation was drastically different than bodies burned in the front and back seats of the same vehicle.

Results of these vehicular fires show that the location of the body within different areas of the vehicle directly influences the extent of heat-related damage and burn patterns to a human body.  These variables should be considered when examining bodies from burned cars, and the death investigator must be aware of how the immediate environment can

be used to anticipate and explain unique burn patterns in fatal vehicle fires, or ones set to intentionally destroy evidence of a crime.

**Human Cremains, Car Fire, Human Remains**

## H116 The Burning Question: A Case Analysis of Peri-Mortem Trauma vs. Post Fire Damage

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After this presentation, attendees will be able to: (1) observe the progression of the pugilistic posture in remains exhibiting perimortem trauma, (2) identify the characteristic features that may indicate pre-existing blunt force trauma in burned long bones, and (3) identify the important points of analysis when investigating perimortem trauma in burned remains.

This presentation will impact the forensic community by outlining points of analysis for the investigation of blunt force trauma to burned remains that will positively assist anthropologists in the correct identification of pre-fire blunt force trauma.

Correctly recognizing and identifying pre-existing perimortem trauma in burned human remains can be challenging especially when soft tissues and bone are destroyed. Despite the damage, it is critical to remember that the distinct characteristics of perimortem trauma can and do survive varying degrees of thermal destruction. Forensic anthropologists must work to correctly separate pre-fire perimortem trauma from thermal damages caused both during and after fire processes. The ability to do these tasks successfully may be compromised by transport of remains to forensic facilities during which time the friability of remains can lead to dramatic fragmentation. Using a recently adjudicated case and a recent case experiment, this presentation will investigate the telltale signs of pre-fire blunt force trauma.

During November of 2007, the Forensic Osteological Investigation Laboratory at the University of California Santa Cruz was called in for blunt force trauma analysis of burned human remains. Of specific concern was the timing of a fracture to the right ulna associated with extensive thermal damage. The posterior portion of the ulnar shaft was completely destroyed and the remaining anterior mid-shaft demonstrated a fracture that extended into unburned bone. In this case, the ulna was analyzed for several categories critical to trauma timing:

1. Fracture Refit
2. Direction of Force
3. Fracture Margin Deformation
4. Color Change

During analysis of these categories, several important features were noted. First, no disarticulation artifacts were noted which suggested that any pre-fire blunt force trauma to the body occurred while the bones were in anatomical relation to each other. Given this fact, the fracture suggested a direction of impact initiating within the interosseous crest which would have been difficult to sustain prior to burning. Second, microscopic analysis of the fracture indicated a close refit of the two fractured sections. Third, the fracture margins lacked deformation which contributed to their close reapproximation. In addition, the changes in coloration due to the thermal damage passed over the fracture line and did not extend into the fractured surface. Given the sum of these determinations it was determined that the defect may have been sustained after the fire due to the fragility of the materials.

Shortly after the completion of the case trial, the opportunity arose to analyze a similar situation experimentally. In June of 2008, the San Luis Obispo country Fire Science Training Program held a forensic fire death investigation course. The course utilized in-class training and burn exercises to teach fire investigators proper fire death investigation techniques. Burn scene 4 consisted of a single adult male cadaver to which perimortem blunt force trauma was delivered to the left radius and left tibia. The fractures were retained completely within the tissue. The burn was recorded via video and thermocoupler for data consistency.

During the course of the burn, the powerful contraction of the arm muscles caused the left radius to pull apart like a hinge, resulting in a stacking of the fractured ends. The complete and unfractured ulna eventually lost articulation with the radial fragments. In the lower limbs, both feet and muscles of the legs flexed completely into the pugilistic posture despite the complete fracture of the left tibia. The right and left distal tibia along with the feet were highly fragmented upon completion of the fire.

Upon examination of the radius it was noted that:
1. If a burned body in the pugilistic pose also shows hinging at a fracture site in the forearm, pre-fire trauma is indicated.
2. Both fractured ends of the radius demonstrated thermal damage and warping and, as a result, the refit was poor.
3. The thermal damage present on the fracture margin extended into the fracture as a result of its exposure to heat from the pugilistic movement.
4. The direction of force required to cause a pre-fire fracture could be readily interpreted by anatomical association of the bones.

This experimental situation reaffirmed the importance of analyzing areas of suspected blunt force trauma in burned remains for quality of fracture refit, suspected direction of force, fracture margin deformation, color change and, if possible, the position of bones and soft tissue following the pugilistic posture. Utilizing these categories as points of analysis during the investigation of blunt force trauma to burned remains will positively assist anthropologists in the correct identification of pre-fire blunt force trauma.

**Fire, Blunt Force Trauma, Trauma Timing**

## I1 A Decision to Withdraw Life-Sustaining Ventilation in a Man With a High Quadriplegic Injury

*Richard Martinez, MD, MH\*, DHMC, 1155 Cherokee Street, Building 18 – Mail Code 3440, Denver, CO 80204*

After attending this presentation, attendees will understand the legal and ethical aspects of withdrawal of life-sustaining treatments in competent patients. Attendees will also learn about depression assessment in the context of competency assessments, and about the ethical and clinical role of the forensic psychiatrist as evaluator and patient advocate in such situations.

This presentation will impact the forensic community by discussing how requests by young conscious patients with high quadriplegia in the first few months after injury present unique and difficult ethical and legal challenges to health professionals and health-care institutions.

While the right to self-determination is established in the ethical-legal traditions governing medical practice in the U.S., withdrawal of life-sustaining treatments is particularly difficult for many health professionals. This is especially true where there are genuine differences of opinion regarding prognosis and quality of life assessments, as well as deeply held convictions about life and death, and who decides. The recent case of Terri Schiavo revealed the intense emotions and divisiveness that such cases can arouse. While the law is clear in the case of patients with irreversible coma and persistent vegetative states, conscious patients requesting withdrawal of life-sustaining treatments present unique legal and ethical issues. How does depression impact decision making? How is competency evaluated? What are the professional obligations to fully inform such patients about future quality of life? The tension between respecting competent patient's wishes and fulfilling professional obligations to do no harm can be fully activated in such situations. In this presentation, an 18-minute video taped interview with a 33-year-old man who requested to have his ventilator withdrawn with full awareness of the outcome of certain death will be shown. This presentation will review the legal and ethical dimensions of requests for withdrawal of life-sustaining treatments, legal aspects of surrogate decision making in such situations, and the complex dynamics involved when such decisions lead to "moral distress" in the health care team responsible for the patient's care. Lastly, the role of the forensic psychiatrist in such a situation will be discussed.

After attending this presentation, attendees will be familiar with the legal and ethical dimensions of end of life decision making involving requests to withdraw life sustaining treatments. Requests by young conscious patients with high quadriplegia in the first few months after injury present unique and difficult ethical and legal challenges to health professionals and health care institutions.

**Medical Decisions, End-of-Life Care, Legal Issues in End-of-Life Care**

## I2 Forensic Typology of Petitioners Requesting Restoration of Firearm Rights

*Kaushal K. Sharma, MD, PO Box 6275, Huntington Beach, CA 92646; and Sidney S. Choung, MD\*, 1815 West Blackhawk Drive, Santa Ana, CA 92704*

The goal of this presentation is to enhance knowledge for psychiatrists regarding firearm restoration rights.

This presentation will impact the forensic community by improving working knowledge when dealing with firearm issues.

Many states including California have enacted laws prohibiting certain group of individuals from owning or possessing firearms. One of these groups of individuals is patients who were placed in mental health facility for involuntary psychiatric treatment. This country has demonstrated a history of public outcry, with little action, after well publicized shootings. However, after the shooting of President Ronald Reagan and his Press Secretary James Brady, the issue of firearm restriction involving an individual with mental illness (John Hinckley) once again became a topic of national debate. Mr. Brady's wife, Sarah Brady, led the organization, Brady Campaign to Prevent Gun Violence, and demanded restricted access to firearms for mentally ill people. Subsequently, federal law was created to deal with such restriction. Existing laws were further strengthened at the federal level due to another outcry after shooting incident by a seeming mentally ill person (Seung-Hui Cho) at Virginia Tech University, killing 32 people.

In California, a person can be placed on an involuntary hold for 72 hours, if he or she is a danger to others, danger to self, or gravely disabled at the time of admission to the designated facility. The paperwork is generated and sent to the California Department of Justice where those individual names are entered in a computer database with a restriction to firearms for 5 years. The individuals restricted under this law can file a petition with the Superior Court of California requesting the restriction be lifted sooner than 5 years.

In the county of Los Angeles, the biggest county by population in California, such petitions are centralized to the Mental Health Department of Superior Court 95. In the Department 95, each one of these petitioners is required to undergo a psychiatric examination and the examining psychiatrist relies on medical records, legal documents (i.e., rap sheets), and face-to-face interviews. As far as is known, other counties do not require such examination.

Based on hundreds of these interviews, it has been learned that petitioners can be divided into the following four distinct categories:

(i) *Make Me Whole*. Individuals who believe their psychiatric hold

was unjust and wrong. It has made them "something less than whole." These individuals believe by having their rights restored the wrongfulness of hospitalization will be corrected and they will become "whole again";

(ii) *I Need My Job*. These individuals might be law enforcement officers, military personnel, armed security guards, etc. Some individuals may pursue employment in such fields in their future;

(iii) *My Guns Are Collectables*. Individual who were taken in due to psychiatric hold were deprived of their "collection" of firearms and these firearms have tremendous sentimental value to them. For example, a rifle from a civil war era handed down from multiple generations; and

(iv) *I am an American*. Individuals who are of strong opinion that they have the constitutional right to bear arms and no court or mental health system has the right to deprive them of their rights to bear arms. These individual are usually strongly opinionated.

In this paper, the implication of these categories will be discussed as well as the likely outcome of petitions and many additional characteristics of these petitioners.

**Firearm Rights, Gun Control, Dangerousness**

## I3 Asperger's Syndrome in a Forensic Context

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After this presentation, attendees will understand the criteria and common symptoms of Asperger's Syndrome. They will also learn to recognize how some of the social and other difficulties common to this syndrome can contribute to activities that manifest as criminal acts. Attendees will learn how these actions can be evaluated in the context of Asperger's Syndrome, with an emphasis on understanding issues of fitness and criminal responsibility.

Asperger's Syndrome is an important diagnostic phenomenon which should be considered more frequently and is in need of much more vigorous research for the future. This presentation will impact the forensic community by contributing to the currently meager literature on the legal implications of Asperger's Syndrome on criminal cases; this information is important both for mental health personnel and all members of the court process.

Asperger's Syndrome is a debilitating neurodevelopmental disorder characterized by impairments in socialization and the presence of repetitive behaviors and focused interests. In particular, persons with Asperger's have difficulty forming and navigating social relationships, and are deficient in their ability to appreciate others' viewpoints. A person with Asperger's that is being tried for a criminal act presents special challenges for the court, as these particular deficits must be taken into account. This discussion will review the current understanding of Asperger's Syndrome and explore its history, diagnosis, treatment options, as well as offer recommendations regarding assessment and management of those with Asperger's Syndrome within the court, jail, and prison systems. Epidemiological information on rates of violence and rates of institutionalization will be discussed. In addition, the presentation will describe the interesting interface of this disorder of socialization and the legal system, with a focus on criminal responsibility and issues of fitness. Conclusions are drawn from specific examples, with a particular emphasis on determining when it is reasonable to consider criminal acts to have been committed in the context of the deficits of Asperger's Syndrome, and when there is no relationship.

**Asperger's, PDD, Psychiatry**

## I4 Domestic Violence and Woman Shelter Houses: A Cross-Sectional Study From Turkey

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After attending this presentation, attendees will understand Turkish legislative regulations for domestic violence cases, the role of the forensic medicine specialists' as physical, gynecological, and psychiatric examinations in the domestic violence cases and woman shelter houses, and coping mechanisms in domestic violence cases.

This presentation will impact the forensic community by providing understanding about the coping mechanisms and woman shelter houses in Turkey.

Domestic violence is a global public health problem all over the world. Forensic scientists have a mandatory duty to participate in these cases. These duties vary from physical, gynecological, or mental examinations, to the autopsy. Many countries have prevention programs against domestic violence or attempt to minimize physical or mental damage to the victim. The Republic of Turkey administers legislative regulations and provides for women's shelters, comprehensive crisis intervention centers, and other measures.

In the presented study, the impact of perception, regarding social support and coping strategies, on the psychological health of women staying in domestic violence shelters was examined. Two different sets of women are compared with respect to their coping mechanisms, their perception of social supports, and their psychological health. The sample size of the research was 107 women, of whom 53 resided in domestic violence shelters and the remainder formed the control group. Participants were given a survey involving tests such as Ways of Coping Inventory (WOC), Multidimensional Perceived Social Support Inventory (MPSSI), Symptom Check List (SCL90-R), questions concerning their violent experiences, and demographic details.

Women staying in domestic violence shelters were found to have significantly higher WOC and SCL-90-R scores, and lower MPSSI scores, when compared to the control group. Emotion focused coping strategies of women staying in shelters, and subscales of SCL-90-R, such as anxiety, depression, and inter-personal sensitivity were found to be related to each other. No significant differences were found in the MPSSI between two different Friend Support, two different Family Support (both top and bottom values), and two different A Special Person score with respect to SCL-90-R subtest scores. No significant relation was found between their perceived social supports, violent experiences, and utilized coping mechanisms. However, a considerable difference was found between the women's violent experiences before marriage and SCL-90-R interpersonal sensitivity and additional items subscales.

In this presentation Turkish legislative domestic violence regulations, system of shelter services, and victim impact of the shelter houses will be introduced.

**Domestic Violence, Coping Mechanism, Turkey**

## I5 Unintentional Injuries Mortality Trends in Puerto Rico, 1999 - 2007

*Magdalena López, MS*, Puerto Rico Department of Health, Epidemiology Office, PO Box 70184, San Juan, Puerto Rico 00936*

After attending this presentation, attendees will be able to study and assess the mortality trends of unintentional injuries in Puerto Rico from 1999 to 2007.

This presentation will impact the forensic community by showing the profile of fatal unintentional injuries for the general population in Puerto Rico. This information allowed the identification of at-risk groups that could most benefit from effective interventions. Prevention effort decisions should be based on data on non-fatal and fatal injuries to better understand the scope of the injury problem and its potential effect on society.

Fatal injuries are an important public health problem in the United States. However, the information about the mortality trends of unintentional injuries in Puerto Rico (PR) is limited. This study assessed the mortality trends of unintentional injuries in PR from 1999 to 2007. The data presented were obtained from the unintentional injuries investigated by the Puerto Rico Institute of Forensic Sciences. For this study, total injuries included deaths related to motor-vehicle traffic, accidental poisoning, falls, drowning, electrocution, and burning.

Descriptive statistics were used to characterize the study population. Mortality rates were age-adjusted to the 2000 standard PR population. Census population estimates were used as denominators in death rate calculations. Mortality rates and trends were stratified by sex and age. Joinpoint regression was performed to determine statistically

significant changes in trends from 1999 to 2007. The Annual Percent Change (APC) from 1999 to 2007 in death rates were calculated for overall unintentional injuries and for the three most common injuries categories: motor-vehicle traffic, accidental poisoning, and falls.

From 1999 to 2007, 11,386 people died from unintentional injuries in Puerto Rico. The annual mean of unintentional injuries was 1,265 cases per year. The overall unintentional injury annual mortality rate decreased from 34.1 in 1999 to 28.8 per 100,000 population in 2007 representing a significant decrease of 5.3%. During the study period, 49.7% of all unintentional injury mortality were caused by motor vehicle traffic; followed by poisoning (25.7%), falls (16.1%), drowning (4.8 %), electrocution (1.4 %), burning (1.4 %), and others (0.9%). For all categories of unintentional injuries men had higher rates as compared with women. During 2007, the unintentional injury mortality rate for males was 4 times the rate for females (46.9 per 100,000 population versus 11.9, respectively).

During the study period the mortality rates caused by motor-vehicle traffic annually decreased 2% (APC statistically different from zero). For both males and females, the motor-vehicle traffic mortality rate was highest among persons aged 15-24 years and people older than 75 years. From 1999 to 2007, the accidental poisoning mortality rate declined (APC: -8.95%, statistically different from zero), while falls mortality rate increased (APC: 13.14%, statically different from zero). For both males and females, the poisoning mortality rate was highest among persons aged 20-54 years.

The falls mortality rates increase proportionally with age. Falls mortality rates tended to increase exponentially in people older than 54 years. This study showed the profile of fatal unintentional injuries for the general population in Puerto Rico. This information allowed the identification of at-risk groups that could most benefit from effective interventions. Prevention effort decisions should be based on data on nonfatal and fatal injuries to better understand the scope of the injury problem and its potential effect on society. Further research is needed to identify risk factors that can decrease the unintentional injuries mortality rates. Integrated prevention programs (e.g., monitoring the risk factors of motor-vehicle traffic crashes, the prevention of falls in older people, and social support services to help people with drug abuse problems) might help reduce the unintentional injuries mortality rates of in Puerto Rico.

**Injuries, Mortality, Trends**

# I6 Serial Killer Luis Alfredo Garavito Cubillos (Colombia, 300 Victims): Self-Perception, Problem Solution Picture Tests, and Childhood Influences

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The goal of this presentation is to show that: (a) even a killer with a very high number of victims (and a supposedly "clever" modus operandi in many locations all over the large country) is not necessarily intelligent, yet extremely street smart, (b) the offender was influenced by typical social factors that are often found in criminals (severe abuse, drugs, etc.), (c) he credibly claims that he misses his substitute family (a woman and her son whom he both took care of in a non-sexual way), and (d) that in his current self-image (aided by a priest who baptized him), Garavito feels that he is now a reborn Christian who will not kill again.

This presentation will impact the forensic community by discussing this unusual case with a very high number of victims.

As a follow-up to the previous, mostly criminalistic case report

(*Proc AAFS* 9:290-291 (2003)) on homosexual pedophile sadist Luis Alfredo Garavito Cubillos (\*1957, Colombia; 300 victims between 1992 and 1999), additional interviews and tests were performed, focusing on his self-perception, intelligence, and childhood influences on this behavior. These are the only known tests ever made in his case.

Several of the factors that Gravito suffers from that are typical for his severity deviant personality structure (paraphilia) are: extreme physical and verbal abuse (violence) by his father, early sexualization (first homosexual relation at the age of 12) and pedophilia, and long term abuse of alcohol. Further findings and observations were picture tests indicate that his intelligence is rather low. He may, however, not have understood the (extremely simple) test instructions due to a lack of education in schools. Gravito suffers from narcissistic and antisocial personality disorders. He shows (or states that he feels) symptoms of schizophrenia like auditory hallucinations (imperative voices) and delusions of being controlled by an external force. Whenever possible, Garavito denies the sexual abuse of his victims because he cannot integrate this particular aspect of his fantasies into his self image. Therefore, and with the help of a local priest, Garavito now projects his feelings of guilt into demons and the devil. Being baptized in prison seems, for him, to heal the personality traits that caused the killings.

The chance of becoming repeatedly delinquent increases with the number of such factors (e.g., German: "kriminogene Faktoren"). Garavito is an example of a classical combination of such factors. This study argues that his personality and the influences in his childhood in their specific combination made Garavito heavily prone towards one of the most intense series of killings in modern times.

Since neither therapy nor picture tests were ever performed on (or with) Garavito before, some of his very straight original statements are additionally presented with letters, drawings, and picture test results which – due to his unawareness of such tests – he could hardly manipulate.

**Serial Killing, Paraphilia, Pedophilia**

# I7 A BRACE Character Profile Analysis of Serial Killer Graham Young

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After attending this presentation, attendees will learn how BRACE can be used to elucidate pathology in the cognitive, behavior, and existential domains of an individual. In particular, the BRACE Character Profile will be used to explore the characteristics of a convicted serial killer.

This presentation will impact the forensic science community by exploring in detail the results of the BRACE analysis of Graham Young.

Graham Young was born on September 7, 1947. He was described as a bright child who had serious difficulties emotionally interacting with others. Although Young was able to approach others, he was a secretive person and tended to interact with others on his own terms. By late childhood, he developed unusual interests in toxicology, especially in the process of dying secondary to poisoning. By the time he reached age 14, Young had also developed other unusual interests associated with death. Young began experimenting with poisoning people with members of his own family. For example, he administered antimony to his father and belladonna to his only sister. Later he began poisoning a classmate after the classmate had fallen out of favor with

Young. The non-lethal poisoning of his classmate and his family members led to Young's arrest and confinement in Broadmoor, a forensic hospital, at age 14. He was released from Broadmoor nine years later.

Young's interests soon branched out to include a fascination with Adolph Hitler and the Nazi movement as well as Dracula. After his release from Broadmoor, Young soon found employment in a company that dealt with chemicals. Within a short time, he obtained poisonous chemicals and resumed his activities involving the poisoning of others. He killed two co-workers by thallium poisoning. Authorities arrested him soon after he suggested to others that the cause of their deaths had been thallium. After his arrest, Young acknowledged having killed his two co-workers. In 1972 he was convicted of their homicides. Although Young was never convicted of other homicides, he later acknowledged having killed his stepmother via poisoning when he was age 14. Graham Young died in prison at the age of 42, reportedly from a heart attack.

In this presentation an overview of the life of Graham Young is provided. The main objective of this presentation is to analyze Young using Behavioral Relativity and Cognitive Economics (BRACE), a sophisticated model of human nature based on the basic principles of learning. The BRACE Character Profile is a 75 item 5-point rating scale based on core aspects of human nature, which includes the following: patterns of maladaptive thought, behavior, and motivation. It is an indirect profiling technique designed to profile any known or well conceptualized individual or prototypical type, real or fictional, dead or alive. The ratings are entered into a spreadsheet to automatically generate graphic profiles and correlations with any other character or prototypical type in the database, including the Personality Disorders (as defined by the current edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders), and a prototypical 40-point Psychopathy Check List-Revised. The wealth of information available for analysis is applied according to the interests of those obtaining the profile. In the case of Graham Young, the analyst was blinded in answering questions related to empathy, intimacy, flexibility vs. controlling, and diagnostic considerations. The results of the BRACE analysis of Graham Young are explored in detail in this presentation.

**Serial Killer, Personality Disorder, Asperger's Disorder**

## I8 Why Do Mothers Abuse and Neglect Their Children? A Four-Pronged Model for Conceptualization, Risk Assessment, and Treatment

*Vivian Shnaidman, MD\*, Jersey Forensic Consulting, LLC, 181 Cherry Valley Road, Princeton, NJ 08540*

The goal of this presentation is to define the four reasons which cause mothers to abuse or neglect their children and help the forensic examiner conceptualize these categories and utilize them in risk assessment and treatment recommendations for the courts.

This presentation will impact the forensic community, as well as all forensic evaluators and investigators who work with abused children and abusive parents and adults. By understanding the typography of the abusive mother, assessments and treatment protocols can be formed which can be recommended to the courts. Likewise, meaningful risk assessments can be performed which will help to prevent reunification of children with parents who are highly likely to be abusive again in the future.

The popular literature about child abuse was examined. While poverty, ignorance, lack of education, and immaturity are often correctly proposed as factors involved in child abuse and neglect, most studies look only at these environmental factors and not at the factors in the mothers themselves. In the presented study personality, emotional,

cognitive, and behavioral factors will be investigated and categorized into diagnostic terms which are easy to understand and to explain. Therefore, a systematic way of approaching these cases will be proposed. The courts respect and rely upon expert testimony in cases of child abuse and neglect, so a definitive paradigm for understanding risk for child abuse is long overdue.

Forensic evaluations for the family courts in New Jersey were examined. Most psychological and psychiatric reports for the courts have numerous features in common. These include certain demographic data, objective information about the case, the subject's own understanding, and putative or working diagnoses, in addition to specific recommendations. Frequently the recommendations are not unique. For example, in almost every case of physical abuse against a child, anger management training was recommended. However, without a solid understanding of the underlying diagnosis and an interdisciplinary approach to treatment, many of these routine interventions become meaningless. Therefore, there is a need to understand the underlying problems which lead to difficulty in controlling the outward expression of emotion and categorize and treat abusive mothers specifically for their own pathology.

In this recent work, the four reasons for child abuse that have emerged are: mental illness, mental retardation or other cognitive impairment, substance abuse, and psychopathy. The topic of cultural factors, such as honor killings, will be touched upon in this presentation, but merits an entire presentation of its own.

In order to evaluate and solidify the hypothesis, written psychological evaluations of abusive and neglectful mothers are in the process of being examined. Correlation coefficients between the existence of abuse and/or neglect to these four causative factors will be investigated and it is hypothesized that the more of these factors that are present in an individual mother, the more extensive the abuse and neglect. Additional statistical analysis will be utilized to estimate the significance of having one or more of the specified conditions. In the future, a model will be derived that can be used to estimate risk of future abuse or neglect against children (similar to risk assessment for violence or sexual violence). The results of the data collection and analysis are expected to fully support the anecdotally observed hypothesis.

Risk assessment for abusive mothers can therefore be understood with a four-pronged approach and this will assist the courts in assessing cases of child abuse and neglect in ways that will protect the children yet preserve the parents' rights to parenthood. This research is seminal in its applicability to various types of abuse against children by their caretakers, by helping both to conceptualize the reasons for abuse in a systematic way, as well as by deriving an actuarial-type instrument which can be utilized in risk assessment and planning for reunification or parental rights termination. The practice of forensic psychology and forensic psychiatry frequently revolves around family law and violence against children by adults. A standard way to approach cases of child abuse, vis-à-vis the courts, can begin not only to explain past behavior but also to predict and prevent future behavior.

**Child Abuse, Female Psychopath, Abusive Mothers**

## I9 Beyond Peer Groups: Kids in Cults

*Thomas R. Hoffman, MD\*, University of Colorado, Denver CARES, 1150 Cherokee Street, Mail Code 3440 – Building 18, Denver, CO 80204*

After attending this presentation, attendees should be able to understand the various definitions of cults, characteristics of cult leaders, methods used by cults to indoctrinate adolescents into the cult, and potential timing and avenues of treatment for adolescents in cults.

This presentation will impact the forensic community by examining the issue of cults and how adolescents are indoctrinated into the cult culture is important so that better decisions about when to intervene and

how to intervene successfully to prevent harm to the adolescent can be made. In addition, it is important for clinicians to be able to identify youth that may be at risk for cult involvement or identify adolescents that may already be involved in cult activities. While there have been numerous cults that have attracted national attention, the vast majority of cults do not do so. It has been estimated that the upper number of cults in the United States approaches five thousand. These organizations will tend to actively recruit people in their teenage and early adult years as this is the time in which recruitment is most successful. It is also estimated that up to fifty percent of high school aged children have been approached to join a cult. It is important for clinicians to understand the high prevalence of cult activity in order to be effective in detecting cult activity. In addition, understanding the social environment of children that have grown up in cults also can be useful in providing effective treatment that can allow for reintegration into society.

Leaders of cults have certain characteristics which allow them to exercise certain command over their subjects. Pseudologia fantastica has been used to describe a set of criteria that may help identify the cult leader and provide an understanding of factors that may allow any person to become ensnared by a cult.

In the course of this presentation, a model for cult progression is reviewed to help the clinician understand the different levels of cult participation. In addition, entry points for intervention, likelihood of success for intervention, and possible intervention techniques are discussed. These points are correlated with the progression of cult involvement. Characteristics and the knowledge base needed for the treating provider are also discussed.

Cults are a pervasive but not always recognizable element of society. While some cults gain national notoriety, most cults operate below recognition. Understanding what constitutes a cult, the personalities of the leaders, the traits of those attracted to cults, progression of cult involvement, and potential avenues for treatment are important topics for the clinical forensic practitioner to understand and to be able to recognize.

**Cult, Adolescent, Intervention**

## I10  Can the Presence of Psychopathy Constitute a Diminished Capacity Defense?

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After attending this presentation, attendees will have a better understanding of the concept of psychopathy with an emphasis on the neurobiology, acquired psychopathy, and the psychiatric legal arguments for and against applying the principles of diminished capacity to psychopaths who commit crimes.

This presentation will impact the forensic community by helping behavioral scientists, criminologists, and the legal communities understand more about the condition of psychopathy and the controversies that it presents.

An argument can be made that psychopathy is a well defined and serious mental disorder with increasingly replicable evidence of neurobiological dysfunction. There is increasing scientific evidence that individuals with psychopathy like those with autistic spectrum disorders (ASD) may have abnormalities in the neurobiological substrates of empathy. Various brain structures and systems have been implicated. This includes the "paralimbic system" consisting of the cingulate, orbitofronal cortex, amygdala, the parahippocampal region, the anterior superior temporal gyrus, and the insula mirror neuron systems. The difference between ASDs and psychopaths appear to be at least in part, that ASDs cannot recognize another person's emotions or empathize whereas the psychopath is able to recognize the emotion and pain of the

other but is unable to feel empathic. For the psychopath, the knowing (cognition) of another person's emotion may simply be just another piece of information like the physical build, spatial details, or if they are armed or unarmed, information to be used by the psychopath in a self serving manner

Is it fair for society to punish individuals who lack the neurological "hardware" for empathy and moral reasoning for their acts in the same manner that society punishes an individual who knows and appreciates that an act is immoral and cold hearted, but chooses to still do so?

Can the presence of psychopathy constitute a diminished capacity/responsibility defense?

Psychopathy presents a challenge and an opportunity for the forensic psychiatrist to look at this complex issue. Advances in imaging genomics and molecular genetics may help establish that in some psychopaths, a finding of diminished capacity or responsibility may be appropriate. Demonstrating the overwhelming deterministic effects of genetic and developmental insults may compel against punishment in a traditional sense. A pragmatic viewpoint is that even if psychopathy is the result of "hard" determinism, wrongful conduct by psychopaths should not go unpunished since it does not result in bad behavior in every instance.

However, science may make it difficult to ignore statistically robust findings linking violence and criminal offending to brain and genetic abnormalities. Evidence that some groups of individuals are "built" differently in a way that causes them to think, feel, and act differently may compel us to consider that they not be treated or punished the same. Acknowledging biological determinism currently impacts how the criminal justice system deals with the mentally retarded, the young, the demented, and even those with chemical and behavioral addictions

**Psychopathy, Limbic System, Diminished Capacity**

## I11  Gender, Personality Disorders, and Intimate Partner Homicide: An Unusual Case of a Murder by a Woman

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After attending this presentation, attendees will be better able to understand the dynamics which play a significant role in the commission of an intimate partner homicide.

This presentation will impact the forensic community by illustrating the interaction of personality traits and disorders with gender specific characteristics related to intimate partner homicide.

Intimate partner homicide is, by definition, a murder which takes place within the context of a dyadic relationship. These crimes are usually carried out by men, as confirmed by the literature, with only a very small percentage being perpetrated by women, who are generally the victims in such cases. In order to offer a better perspective on the representation of this phenomenon, it may suffice to report that studies conducted by various Italian statistical agencies have found that in 90% of these types of cases, men are the ones to murder their female partners.

According to the literature there are also other common features shared by male perpetrators of these crimes. For example, there is often a very high level of violence carried out in these cases. Another common feature is that a significant age disparity often exists between the man and the woman, with the man usually being the older of the pair. But when the perpetrator is a woman, the dynamics are usually quite different; most notably there is a much lower level of violence associated with the homicidal act, and it is usually the final reaction to a history of

battery and abuse. In addition, when the perpetrator is a woman, she is usually the younger of the two.

The unusual case presents features which are more consistent with those characteristics associated with male perpetrators, often described in the literature. The only difference here is that the perpetrator was a woman, and the victim, her younger boyfriend.

The most striking and immediately apparent feature of this homicide is the level of violence associated with the act, which resulted in overkilling. The victim was found with multiple stab wounds (38) which were located at the neck, thorax, abdomen, and hips some of which had been inflicted postmortem.

The female perpetrator was 51-year-old and the male victim 28-year-old. They had an intimate relationship over a two-year period. The woman, who was previously married, left her husband to live with her new and much younger boyfriend.

The homicide took place in her bedroom, soon after the boyfriend had informed the woman of his decision to leave her to marry another woman, several years his junior, and with whom he had been having a simultaneous affair for a number of years. It was at that point the woman, who had been deeply committed to the relationship, and who was unable to tolerate her sense of frustration and feeling of abandonment, displayed an outburst of violence with the goal of destroying the object of her love. In an attempt to symbolically annul the abandonment and concretely make it impossible for him to leave her for a much younger woman, she went to the kitchen and returned to the bedroom, where her partner lay waiting for her and repeatedly stabbed him with a knife as he tried to defend himself and escape from the bedroom.

The dynamics described here are usually associated with males who kill their partners after realizing that that their significant others will no longer remain with them. It is highly uncommon that such a modus operandi is carried out by a woman with such an exaggerated level of violence.

Following psychiatric evaluation, the subject was diagnosed as having a borderline personality and narcissistic traits. Psychodiagnostic testing (Rorschach, O.R.T.) was administered, illustrating a tendency toward regression and the use of projective defences, autistic fantasies, affective inadequacy, fragility of the Ego, ambivalence, high impulsivity, and a tendency toward aggression.

These personality characteristics appear to be similar to those of male perpetrators who have committed intimate partner homicide in the same manner, in similar contexts, and with similar precipitating factors.

In light of these considerations, the hypothesis could be put forth that personality organization may play a significant role in similar homicidal acts, and that in some cases, personality organization may also be more significant than gender.

**Intimate Partner Homicide, Personality Disorders, Gender**

## I12 Male Victims of Sexual Assault: Involuntary Erection and Ejaculation

*Clayton M. Bullock, PhD\*, Institute of Psychiatry Law and Behavioral Science, USC, LAC + USC Medical Center Psychiatric Outpatient Clinic, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will be educated on the incidence and prevalence of the sexual assault of adult males in non-incarcerated settings, the motivations of the perpetrators of these crimes, and the phenomenon of involuntary sexual responses on the part of victims. Sexual responses (erections and or ejaculations) are frequently misinterpreted by victims, assailants, and the criminal justice system alike as signifying consent. The physiology governing these responses is reviewed and supports the view that men can experience erections and ejaculations under a variety of adverse circumstances, including during an anal rape.

This presentation will impact the forensic community by detailing how men can have erections and ejaculations under adverse circumstances, including during a sexual assault. The presence of an erection or the occurrence of ejaculation does not necessarily signify consent. Attempts to cite the occurrence of an ejaculation or erection as evidence of consent by the victim are unwarranted.

Although males are much less frequently victims of sexual assault than are females, sexual assault of males is by no means a rare phenomenon, nor is it limited to all-male populations such as jails and prisons. Retrospective data from large randomized community samples estimate a prevalence of male sexual assault victimization of between 3 and 7%. As with females, sexual assault of males occurs more frequently in the victim's 20s or 30s. Where comparisons between male and female victims are available, it appears male and female victims are assaulted by strangers at about the same rate, but that males may more likely have more than one assailant. The studies that address the sexual orientation of male victims find higher percentages of victims who identify as gay, bisexual, or having consensual sex with men. However, these populations are more highly represented in the samples of the studies where sexual orientation is addressed. Many male assaults involve anal rape.

The circumstances in which rapes of men take place are varied. As with women, men can be assaulted by acquaintances (including recent acquaintances), lovers, friends, family members, and by total strangers. These assaults take place in the community as well as in jails and prisons. The motivations of the assailants are varied, and include demanding sexual gratification from a lover, partner or recent acquaintance, exorcising intensely conflicted feelings about sexual orientation, and the humiliation of and exercise of power over the victim. An extreme form of power is expressed in the victim's becoming aroused or ejaculating during an assault. This is a frequent occurrence, which is incorrectly understood by assailant, victims, justice system, and medical community as signifying consent. What is understood about the physiological mechanisms underlying involuntary erection or ejaculation suggests that these can and frequently do occur in the context of non-consensual receptive anal sex. Erections and ejaculations are at best only partially under voluntary control and can take place even during times of extreme stress or duress.

Men are even less likely to report being sexually assaulted than are women. Reasons for this under-reporting include fear of stigmatization, fear of being labeled homosexual, fear of being disbelieved and further stigmatized by law enforcement officials and health care providers, and shame and disbelief over the loss of control that the sexual assault may represent to the victim. Having an erection or ejaculating represents an extreme form of loss of control, and may further dissuade the victim from reporting the assault. Indeed, assailants frequently attempt to manipulate their victims into ejaculating as a strategy for further humiliation and a deterrent to the victim's reporting the crime. The criminal justice system has historically been unavailing of male victims of sexual assault, particularly when the victim experienced an erection or an ejaculation.

This presentation reviews studies that examine the incidence and prevalence of these crimes, the motivations of the assailants, contexts in which these crimes take place, and the underlying physiological mechanisms governing erections and ejaculations, including the recently identified "ejaculation generator" in the spinal cord. It is argued in this presentation that men can have erections and ejaculations during an assault and that these do not necessarily signify consent.

**Anal Rape, Male Rape, Involuntary Ejaculation**

## I13 General and Forensic Psychiatrists as Objects of Inimical Conduct

*Alisha R. Smith, MD\*, USC Psychiatry and Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will become aware of the prevalence of inimical acts against general and forensic psychiatrists and the impact of this behavior on psychiatrists. Attendees will learn how to identify potential offenders and when dangerous behavior is escalating. Lastly, they will understand the steps to take if they are being victimized.

This presentation will impact the forensic community by heightening the awareness of the frequency of attacks against general and forensic psychiatrists. Also by teaching psychiatrists how they can protect themselves by identifying potential attackers, identifying escalating dangerous behavior, and teaching appropriate measures to take in order to preserve personal safety.

Personal safety is an issue that concerns everyone. In the general population as many as 2 out of every 25 women and 1 out of every 50 men have been the victims of inimical behavior. Moreover, general psychiatrists and forensic psychiatrists are at great risk for stalking, assaults, and violent threats. Literature has reported that up to 1 in 5 general psychiatrists has been stalked, harassed, threatened, or assaulted by their patients and up to almost half of forensic psychiatrists have experienced such behavior. Therefore, mental health care professionals need to be aware of perilous behavior.

Threats to personal safety can have a severe impact on the psychiatrist's emotional, occupational, and social functioning. The impact can vary from simply changing one's daily routine to having to changes jobs and/or move to another city. Often, the longer the perpetrator demonstrates the deviant behavior, the more upsetting and disruptive it becomes to the victim. In the most severe cases, psychiatrists had to be treated for post-traumatic stress disorder.

The statistics regarding imperiling conduct are alarming. How can psychiatrists guard their personal safety? Psychiatrists need to be aware of how to identify possible offenders and recognize when their behavior is becoming more dangerous. Psychiatrists need to be on high alert if the patient/client has stalked or harassed before and/or has had a violent history. Perpetrators tend to be male and have a diagnosis of personality disorder, substance abuse disorder, schizophrenia, and/or mood disorder. Indicators of escalating dangerous behaviors include: instances when the patient/client becomes more obsessed/fixated on the psychiatrist resulting in more contacts, demonstrates an increased degree of negative emotions (e.g., anger, jealousy, hatred) toward the psychiatrist, and makes an increased number of threats.

The most appropriate measure for a psychiatrist to take if they are being victimized is to avoid direct contact with offender. Often victims of adverse behavior want to confront the offender. Unfortunately, confrontation encourages more of the unwanted conduct. The police should be immediately notified and the psychiatrist should notify their supervisor and team at their job. The supervisor and team can aid in acting as a barrier between the psychiatrist and the offender. Psychiatrist should promote their own personal safety by enhancing alarm systems, varying daily routines, and investing in a personal security officer, if needed. Most importantly, document and record any communications the perpetrator has made which will help the case when filing for a restraining order and bringing criminal charges, if required.

In summary, mental health professionals are at risk for being victims of adverse behavior. The impact that victimization has on the psychiatrist's psychological, occupational, and social functioning can be severe. It is important that psychiatrists are aware of this fact and can identify possible offenders as well as know if an offender's dangerous behavior is escalating. It is imperative that psychiatrists know how to protect their personal safety if they are objects of inimical conduct.

**Stalking, Assault, Psychiatrists**

## I14 Special Topics in Forensic Addiction Medicine: Relevancy, Criminal Law, and Adolescent Populations

*Dean De Crisce, MD\*, 41 Schermerhorn Street #325, Brooklyn, NY 11201; Richard Rosner, MD\*, Forensic Psychiatry Clinic, 100 Centre Street, Room 500, New York, NY 10013; and Gregory C. Bunt, MD\*, Daytop Village, 54 West 40th Street, New York, NY 10018*

After attending this presentation, attendees will be able to recognize the usefulness and applicability of addiction expertise in the forensic behavioral sciences. Attendees will also learn relevant case law affecting legal decision-making in the context of addiction and intoxication, and utilize interventional and treatment strategies directed towards adolescents with substance use disorders.

This presentation will impact the forensic community by encouraging expertise in addiction medicine, particularly as it applies to forensic behavioral science. This will include a discussion of the relevancy of such expertise, history of case law development in the field, and applicability of treatment concepts in a specific, unique population.

Substance use disorders and intoxication are pervasive in mental health and criminal populations. Up to 80% of incarcerated and arrested populations in the United States have had a history significant for substance use disorders. Community costs exceed hundreds of millions of dollars each year which are directly attributable to drug and alcohol use. Creation of special drug courts within many jurisdictions has been initiated, in part, because of the pervasive nature of substance use disorders within the judicial system.

Substance use disorders, significantly debilitating illnesses, are associated with violent crimes, drug diversion, child abuse, exacerbation of mental illness, personality disorders, poor social functioning, poverty, domestic violence, and multiple causes of unexpected death. They are predictive of a high rate of criminal recidivism, and increase risk factors such as disinhibition and aggression. Substance abuse treatment has been shown to decrease criminal behavior, morbidity, and violence.

Despite these facts, there is little professional training in addictive disorders offered to behavioral clinicians and forensic scientists. Specialized proficiency in the addiction field is even less common. Therefore, expertise in addiction medicine is a valid and important goal of training within the field of forensic behavioral sciences. As the subject has broad ramifications involving aspects of presentation, prognosis, etiology, treatment, evaluation, behavioral correlation, and interaction with the legal system, three useful topics were chosen for presentation.

Dr. Bunt, faculty member of the Division of Alcoholism and Drug Abuse at the NYU School of Medicine, and medical director of Daytop Village, a highly successful therapeutic community, will present compelling evidence for the need of addiction expertise within forensic psychiatry. Understanding addictive disorders and its application enhances the credibility, and effectiveness of the forensic scientist.

Dr. De Crisce, faculty member of the Division of Forensic Psychiatry at the NYU School of Medicine, and a forensic psychiatrist performing risk assessments for child protective services and of sexual offenders will review aspects of substance use disorders which impact on criminal law. Consideration of addiction and intoxication might be involved in assessments of competency, diminished capacity, fitness for duty, parental rights, dangerousness, malpractice, and involuntary commitment.

Dr. Rosner, renowned director of the NYU-Bellevue Forensic Psychiatric Court Clinic, director of the NYU Forensic Psychiatric Training Program, author, and forensic psychiatric leader will discuss special considerations of addiction medicine within the adolescent population. There is a clear relationship between substance use and emotional and behavioral problems, involving delinquent, aggressive and criminal behaviors in adolescents. Much of the United States

incarcerated adult population began problematic behavior in their adolescent years, associated with substance use, which has been subsequently related to adult criminality. Intervention, evaluation, and treatment are crucial to prevent adverse outcomes.

**Addiction Medicine, Forensic Psychiatry, Adolescent Chemical Dependency**

## I15 Juvenile Psychopathy and Development

*Christopher R. Thompson, MD\*, 10850 Wilshire Boulevard, Suite 850, Los Angeles, CA 90024*

The goal of this presentation is to explain psychopathy, explore the validity and utility of this concept in the juvenile population, and examine developmental concerns that arise from application of the concept to youths.

This presentation will impact the forensic community by helping forensic evaluators better predict violent recidivism and general recidivism in juvenile offenders.

Psychopathy is a construct with which forensic psychiatrists are quite familiar. The presence of this "disorder" in adults is one of the best predictors of general and violent recidivism and, to some extent, amenability to treatment. Recently, the concept of juvenile psychopathy has been proposed.

For a variety of reasons, the designation of a juvenile as a psychopath may be problematic. Transient, normative developmental phenomenon, and behaviors may be mistaken for fixed, maladaptive, malignant personality patterns. Since "amenability to treatment" is an important consideration in both the juvenile justice system (and to some extent, the adult criminal justice system), this may lead to lengthy, perhaps unnecessary periods of incarceration for juveniles erroneously identified as psychopaths.

Recent research is attempting to determine whether psychopathy is a valid construct in juveniles and whether its component traits are stable over time. However, this research is in its infancy and has not yet confirmed or disconfirmed the validity or predictive utility of juvenile psychopathy. Hopefully, time will allow the development and employment of instruments that more accurately predict which juveniles are likely to continue offending as adults (particularly violently offending). This should lead to more just legal outcomes for minors, help protect the general public and preserve the dignity and integrity of the legal process.

**Psychopathy, Juvenile, Conduct Disorder**

## I16 What Constitutes Typical Adolescent Behavior and How Is It Different From Adult Conduct?

*Alison G. Vredenburgh, PhD\*, Vredenburgh & Associates, Incorporated, 2588 El Camino Real, F353, Carlsbad, CA 92008*

Attendees to this presentation will gain an understanding of the process involved in conducting original research as part of a behavioral sciences forensic investigation as well as gaining insight into how to evaluate psychological factors that often interact with engineering issues.

This presentation will impact the forensic community by demonstrating an approach to evaluating issues that cannot be addressed using standard site inspection and laboratory techniques. This presentation will discuss typical behavior and expectations of adolescent product users.

In the field of forensic human factors, a relevant question when performing an analysis of an injury incident is: Was the person involved in the incident acting as expected? In the forensic arena, this translates to "reasonableness of conduct" and are behaviors consistent with societal norms for a given population? Age is an important consideration when evaluating reasonableness of conduct because behaviors that are perceived as unreasonable for an adult may be typical for adolescents.

In order to assess typical adolescent behaviors and injury occurrence of injuries, a survey to addressed actual risk-taking behavior in the common adolescent injury categories described above as perceived by the adolescents themselves. This methodology differs from previous studies in the field as the adolescents themselves were surveyed, not their parent or guardian, in determining the level of risk taking behaviors in high school students.

In this research project, there were 144 adolescent participants ranging in age from 13 to 18 with a mean of 14.7-years-old and a SD=1.04. There were 69 males and 70 females (5 did not report their sex) in the study. Their GPA ranged from 1.0 - 4.8 with a mean GPA of 3.23 and a SD=0.88. Overall, 48% of the participants reported experiencing injuries resulting from an accident that required medical treatment.

Participants were asked to what extent they protect themselves by wearing personal protective equipment (PPE) when riding bikes, skateboards and scooters. Table 1 depicts the frequencies and percent of participants who use protective gear. Ninety-five (71.97%) of the participants of these activities report that they wear PPE half of the time or less.

Table 1. Use of protective equipment

| Frequency of use | Frequency | Percent |
|---|---|---|
| Almost Never | 65 | 49% |
| 50% of time | 30 | 23% |
| Most of the time | 27 | 20% |
| 100% of time | 10 | 8% |

Many accidents involving teenagers involve water sports. Therefore, participants were asked if they have ever been injured while diving head first into water without knowing how deep it was; 48% of the respondents reported that they had. They were also asked if they ever ignored posted warnings indicating that it is not safe to swim or dive; 48% reported that they ignored warnings.

Of the 25 (18.4%) participants who drive, 65% drive with a learner's permit and 35% with a license. New drivers and those with a permit are not allowed to drive non-family under 18 years of age. When asked if they have driven other kids when their license did not allow it, 14% of the drivers reported that they did. Of the drivers, 13% reported that they had been in an accident while driving.

Participants were asked if they were injured from accidentally falling from a height or while jumping down from a height. Forty-six (34%) reported injuries after accidentally falling and 31 (23%) were injured after intentionally jumping.

Participants were asked if they ever played with fire and had it get out of their control. Forty (29.4%) respondents lost control of fire and 20 (15%) were burned enough to seek medical care. Eighty-nine (65%) have been with other adolescents who were playing with fire.

Participants were asked if they ever needed medical treatment after being injured while using a consumer product; 45 (39%) reported an injury. Of those injured, most have been injured while using sporting equipment. Table 2 depicts the injury categories.

Table 2. Product-related injuries

| Product | Frequency | Percent |
|---|---|---|
| Tools/Knives | 25 | 48.1 |
| Sporting Equipment | 20 | 38.5 |
| Chemicals | 4 | 7.7 |
| Toys | 1 | 1.9 |
| Other | 2 | 3.8 |
| Total | 52* | 100 |

*The injuries total more than 45 because some participants had multiple injuries.

Participants were then asked how often, if ever, they would read instructions or warnings on products. They were also asked under what types of conditions they would read warnings. Table 3a provides the frequencies that they read instructions and warnings. Table 3b describes situations when they read warnings (for those who read them).

Table 3a. Frequency that participants read instructions and warnings

|  | Always | Sometimes | Rarely | Never |
|---|---|---|---|---|
| **Instructions** | | | | |
| Freq. | 29 | 72 | 24 | 14 |
| % | 20.9 | 51.8 | 17.3 | 10.1 |
| **Warnings** | | | | |
| Freq. | 25 | 64 | 32 | 17 |
| % | 18.1 | 46.4 | 23.2 | 12.3 |

Table 3b. Situations when warnings were read

| Situation | Frequency | Percent |
|---|---|---|
| New product | 60 | 44.8 |
| Looks dangerous | 63 | 47.0 |
| Know someone injured | 11 | 8.2 |
| Total | 134* | 100 |

*Some participants read warnings for multiple reasons

Participants were asked if they had been injured in school. Sixty-five (47%) reported that they were injured enough to require medical treatment. While most of these injuries occurred in PE, sports or lunch, there were two injuries in science class and one that occurred in English class. Nine (6.8%) were caused by fighting. Twenty-eight (20%) reported that they injured other students while fighting. Throwing of objects at students is very common; 113 (82%) of the respondents reported seeing other students throwing objects. Seventy-six (56%) admitted to throwing objects themselves and 31 (23%) said that they had been injured by an object thrown at them.

Of the 158 objects reported to have been thrown by the participant or observed being thrown, the most common objects thrown were pencils and pens (47; 30%), food (26; 16%), paper (24; 15%), balls (18; 11%), rocks (18; 11%), erasers (8; 5%), books (4; 3%), chairs (4; 3%), paper clips (3; 2%), and scissors (2; 1%). Also thrown were a trashcan, knife, bullet, and a shoe.

Overall, there was no significant difference in accident rates between male and female participants. Females were found to be more likely to ski and snowboard than males (t(133)=3.12, p<.01); they were also injured significantly more than males while skiing and snowboarding (t(77) = 2.16, p<.05). Males were significantly more likely to have been burned while playing with fire (t(129)=2.51, p<.05). Males were also injured more in fights (t(135) = 2.18, p<.05) and threw objects at other students more often than females (t(134)=2.45, p<.05). Males reported that they read instructions (t(137)=2.49, p<.05) and warnings t(136)=1.99, p<.05) significantly more frequently than females did.

Despite the fact that the majority of respondents have been injured in one of the investigated categories, half of them almost never wear protective equipment. This indicates adherence to the personal fable that bad things only happen to other people. Similarly, the reported frequency of reading warnings was low.

These results reinforce the notion that what is typical behavior for an adult may not necessarily be the same for adolescents. Although many adolescents are at the cognitive developmental stage to be able to make well-informed decisions, it is the norm for this age group to be strongly influenced by outside forces. Therefore, decisions based on some of these outside forces may not lead to what can be considered typical adolescent behavior.

**Adolescent, Safety, Accidents**

# I17 Involuntary Treatment Without Advanced Directives

*Phani M. Tumu, MD\*, USC Institute of Psychiatry & Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will be updated on the current practices for advanced directives, both medically and psychiatrically.

This presentation will impact the forensic community by examining the various types of advanced directives available.

In 2006, the case of Terry Schiavo put the importance of having advanced directives into the national spotlight. In California, persons without advanced directives are given life-supporting treatments until the medical staff decides that further medical care would be futile. For those without advanced directives and who are not on life-support, what options do primary care providers have available to them?

Currently in California, a medical provider has the legal authority to provide life-saving measures to those whose life is in grave danger, regardless of whether the patient has an advanced directive. In these cases, it is the duty of the physician to provide life-sustaining care. However, in non-life-threatening emergencies, the decision to provide care to those without advanced directives falls into a grey area.

In these cases, the actual meaning of the word "care" should be taken into consideration. What constitutes "care" for a patient who does not have an advanced directive? In the case of Terry Schiavo, who did not have an advanced directive, the "care" rendered to her at the latter stages of her life was a feeding tube which essentially prolonged her life. If looking at a broader definition of care, can a physician, for example, prescribe antihypertensive treatment to a patient? While a medication may be more peripheral to life than the provision of nutrition, this type of treatment could be considered similar to a feeding tube in that both treatments extend the life expectancy of any individual for whom the treatment is given.

It is also worth re-examining the definition of "advanced directive" in those patients who already have advanced directives. It is entirely possible to extend the quality of life of a patient without actually performing invasive techniques, which may not be allowed in the patient's advanced directive. In one study, patients enrolled in California Durable Power of Attorney for Health Care (DPAHC) actually requested less medical intervention. However, the same study showed that having a summary placed in the patient's medical record had no significant positive or negative effect on a patient's well-being, health status, medical treatments, or medical treatment charges.

In a separate study, 68% of the subjects executed the DPAHC. Most patients wished treatments to be limited or withheld under certain conditions of reduced quality of life. Although general instructions noted on the DPAHC and preferences regarding specific procedures were stable over the course of a year, the advance directive's general instructions were often inconsistent with, and poor predictors of, specific procedure preferences. It was concluded that the brief general instruction component of the California DPAHC is not helpful in communicating patient wishes regarding specific life-saving procedures.

California's advanced psychiatric directives are even more complex. In fact, psychiatric advanced directives are accepted in only 14 of the states. Given the complex nature of advanced directives, it is not surprising that most states do not recognize psychiatric advanced directives; however, given the complex nature of mental illness, it would be advisable to have a recognition of psychiatric advanced directives, especially in those patients with the most severe of mental illnesses.

**Advanced Directives, Power of Attorney, Terry Schiavo**

## I18    Fast Tract ECT for the Gravely Ill

*Phani M. Tumu, MD\*, USC Institute of Psychiatry & Law, PO Box 86125, Los Angeles, CA 90086-0125*

After attending the presentation, attendees will be able to rationalize the need for earlier administration of ECT and various proposals on how to apply for court authorization.

The presentation will impact the forensic community by explaining various ways a psychiatrist can enlist a court to help expedite the administration of ECT to those whose condition can rapidly deteriorate, or to those whom are gravely ill already.

In California, extraordinary measures are needed in order to administer electroconvulsive therapy (ECT) for patients who suffer from conditions such as refractory depression and manic-depressive illness. For example, in depression, a patient would have to fail multiple trials of different antidepressants before ECT could be considered. This is in the face of evidence showing that ECT is superior in efficacy to any antidepressant now available. Why is ECT so difficult to administer in the state of California, particularly when physical conditions resulting from these psychiatric illnesses can be significant enough to require medical hospitalization? Should there be a way to obtain consent for ECT before patients become so gravely ill as to require medical hospitalization?

The treatment of depression can be challenging, as the Sequential Treatment Alternatives for Resistant Depression (STAR*D) study demonstrated recently. The STAR*D study is a multi-center study which tested the efficacy of various classes of antidepressants in patients with resistant depression. In the best case scenario, current antidepressants were found to have an efficacy of 35%, in any patient population. This means that only about one out of every three patients will respond to the first antidepressant prescribed to them. A certain amount of time is needed to judge efficacy; if the patient does not respond to the medication, the physician usually switches to a second antidepressant, usually of another class, which may or may not work. In the meantime, the patient is getting possibly worse, not just from the side effects of the failed medication, but also from the natural course of the depression.

However, because depression is an illness associated with significant morbidity, and sometimes mortality, time to treat successfully is of paramount importance. Given the evidence that ECT is an effective intervention for depression, a psychiatrist experienced with ECT should be able to provide such treatment earlier in the course of treatment, especially in those patients with a deteriorating clinical condition. Clearly, the importance of obtaining informed consent should not be overlooked.

Given the historical difficulty in performing ECT, what measures could be taken to ease its administration in light of its demonstrated efficacy? One method would be for the treating psychiatrist to obtain court-authorization, either by direct court testimony or by separate paperwork (similar to a 3200 petition in California, in which a physician requests to have court authorization to perform medical procedures for patients on involuntary psychiatric holds). Another method would be for the doctor to present the case at a hearing in the hospital to a representative of the Court (similar to a probable cause hearing referee in California, a court-appointed official who determines the legality of 14 and 30 day holds). The decision of the Court representative would be final and the doctor would then proceed according to that decision. Such "fast track" ECT could prevent the significant deterioration and possible life-threatening condition associated with depression or other psychiatric illnesses.

**Electroconvulsive Therapy, STAR*D Study, Depression**

## I19    Forensic Psychiatry in Turkey: A Cross-Sectional Study

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After attending this presentation, attendees will understand the Republic of Turkey's forensic psychiatric expert system and learn how to prevent the contradictory opinions in forensic psychiatric cases.

This presentation will impact the forensic community by providing a useful and important forensic psychiatry study.

Forensic psychiatry is a branch of forensic sciences which focuses on the interface of law and mental health. It may include psychiatric evaluation in a wide variety of legal matters as well as clinical work with perpetrators and victims in civil or criminal litigations. The numbers of forensic psychiatric cases are increasing all over the world, day-by-day, as in Turkey. Expert opinion is of vital importance in the trial process, the final decision of the lawsuits.

The Republic of Turkey has approximately 70 million people and, as a result, lawsuit numbers are very high. In Turkey, judicial authorities receive expert opinions about forensic psychiatry from various psychiatric hospitals medical boards. Generally, views obtained from these boards are sent to Forensic Medicine Speciality Committee-IV which is the relevant specialization unit of The Council of Forensic Medicine of Turkey. This committee, the most experienced forensic board with psychiatric expertise in Turkey, reevaluates the referred cases. The committee, in Istanbul, is comprised of forensic medicine specialists, general psychiatrists, child psychiatrists, and neurologists. On occasion, discrepencies regarding the same case are encountered. Of course, expertise evaluations in forensic psychiatry require special experience and approach. In this study, the reasons and frequencies of this discrepancies and consistencies in the same cases were evaluated.

To this purpose, judicial files of cases appealed to Forensic Medicine Speciality Committee-IV between June 1, 2005 and October 1, 2005 were examined and the results given by Committee-IV were compared with reports prepared previously from other expertise boards. Cases were identified randomly but in a certain methodology and measure including demographic and other datas, performed in a face to face interview. While comparing these decisions, variables including positive predictive values, negative predictive values, sensitivity, specificity, and parallel diagnosing were assessed and findings were compared with similar other studies.

As a result, it was identified that the judgements given by Forensic Medicine Speciality Committee-IV for some cases were parallel to judgements given previously for the same cases. There were, however, contradictions for remaining cases. The essence of dispensation of justice is to reflect equal evaluations pertaining to similar matters for everyone. This is important to constitute a society of individuals who live in peace and receive equal in legislative procedures.

In this presentation, Turkey's forensic psychiatric expertise system will be introduced. Secondly, the results of the present study will be disclosed. Finally the prevention of contradictory judgements given by different experts in same forensic cases will be discussed. Perhaps, it is mandatory to follow cardinal principles and rules in all areas of expertise, especially thoses in which concrete data are less available such as psychiatry. For this purpose, identifiying the elements of guidelines and professionals being amenable to these guidelines is a necessity for effective application of justice. Coordination and collaboration of experts is necessesary to elicit this goal.

**Forensic Psychiatry, Expert Witness, Turkey**

## I20   Decision Making in Child and Adolescent Dependency Cases

*Michael S. Tramell, MD\*, USC Institute of Psychiatry and the Law, PO Box 847, Corona Del Mar, CA 92694*

After attending this presentation, attendees will understand the process by which decisions, especially those regarding mental health treatment, are made in the juvenile dependency system.

This presentation will impact the forensic community by demonstrating how decisions are made for children and adolescents in the dependency system.

There are conflicting opinions over who has the ability to make decisions regarding the mental health care of children and adolescents who are within the juvenile dependency system. This paper will clarify who has the legal right to make decisions and demonstrate the processes by which these decisions are made.

Children in the juvenile dependency and juvenile justice systems are frequently treated similarly, although important differences exist between the legal situations of these groups. These differences shape the process by which decisions are to be made for each group. Children in the dependency system are usually there for reasons involving actual or substantial risk of custodial abuse or neglect. This can take the form of risk or actual physical, emotional, or sexual harm, abuse, or damage to the child. Additionally, being the victim of neglect, subjected to cruelty, or being freed by the parents for adoption, also result in the child entering the dependency system. In California, allegations of the above are investigated by a social worker, who may then petition the court for the removal of the child. If at the time it is felt to be in the child's best interest, the court has the option of appointing a guardian to make decisions for the child, or an individual who only makes educational decisions for the child. The court may also choose to limit the parental rights, via court order, in any number of ways. If the child is removed from the parents, they are placed under the supervision of the social worker, who then must place the child appropriately. An assessment by the agency supervising the minor is then prepared for the court, which then must decide whether to terminate the parental rights. If parental rights are terminated, a decision must be made to determine whether to appoint a guardian for the child or place the child in long term foster care. If a guardian is appointed, the court may decide to terminate its oversight, but frequently chooses to retain its jurisdiction. From this point on, reports are required by the court every six months to monitor the child until they are adopted. The court gives the guardian specific rights and decision making privileges. If the guardian is not explicitly authorized to make a treatment decision, the court can make it. Family Code Law states that minors age 12 and greater can request or consent to mental health treatment, however, administration of psychotropic medication to a minor is explicitly forbidden without the consent of the minor's parent or guardian. The standard that the court is required to use in making treatment determinations, or in decision making on the child's behalf, is the "best interest of the child." In dependency court hearings, an agency can be joined to the hearing if the court finds that that agency failed to meet its legal obligations to provide services for the child. However, prior to this the child must be found eligible for services, usually through an assessment as part of an Individualized Educational Plan (IEP). Assessments are made through the IEP process in a variety of specialized fields, including mental health, for the purpose of improving school functioning. When these assessments are made by appropriate individuals, after discussion with the parent or guardian and the IEP team, the recommendations of the assessor become the recommendations of the entire IEP team, including those regarding psychotropic medication. These recommendations serve as a guide for the judge or guardian in making decisions for the child. Mental health treatment teams are frequently involved in the care of dependent children, especially those placed in institutions. These treatment teams may have members who are also on the minors IEP team. However, treatment teams themselves do not have standing to make treatment decisions for minors who are dependents without getting the approval of a judge. An example of this is the direct petition the treating psychiatrist submits for consent for psychotropic medication. This must be approved by the court prior to the administration of any psychotropic medications. Specific suggestions for incorporating the child and adolescent into the process of decision-making will then be discussed. Awareness of the process by which decisions are made for youth who are in the dependency system varies by county and state. Any forensic consultant or clinician involved in their care should be aware of their particular jurisdiction's process.

**Decision Making, Dependent Minors, Mental Health Services**

## I21   Power Fantasies of a Serial Sexual Offender

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After attending this presentation, attendees will compare their knowledge regarding sexual offenders' "modus operandi" with other kinds of scientific experiences.

This presentation will impact the forensic community by explaining sexual offenders' behavior, their criminal and psychological profile, the power of their fantasies, and the meaning of their assaults.

Between September 2002 and November 2005, in two small southern Italian towns several miles apart, nineteen very similar sexual assault charges were filed by young women.

In all cases the following was reported: The victims were young women who were returning alone to apartment buildings located in the outskirts of the town. The aggressor followed them in as they entered the building, then silently approached while taking hold of the victim from behind. The aggressor held them by the hair and threatened them with either a knife to the throat or a gun (toy) to the temple, while whispering to remain silent as he caressed their genital areas.

In only one case was there sexual intercourse with a victim who fearing harm would be brought against her, gave herself to the aggressor after having undressed by herself in a field near the apartment building.

The aggressor vanished at any resistance by the woman. The aggressor did not use brute force or behavior. Although all of the women reported not seeing the agressor's face, all reported the aggressor being young and acting gently.

All the acts took place between 5:00 and 7:00 p.m.

A 38-year-old man married with two children and an attendant for a private clinic was arrested for the charges of sexual assault. When arrested the man admitted not only to the charges but added an additional ten victims that had never been reported in the same towns. He explained that after leaving work he regularly took a walk through the outskirts of town in the hope of finding a suitable female victim leaving his car in the neighborhood for quick escape.

Medical-psychiatric observations of the subject showed narcissism traits in his personality, dissatisfaction in rapports with women, and problematic inter-relational skills. On a clinical level it was found that he suffered from depression, relational anxiousness, and impulsivity. The clinical diagnosis included SCID P and II, BECK AT scale (score: 37), MMPI-2, Rorsach and Barrat Impulsivness Scale (score: 78). The PCL-R test by Hare (result: <20) excluded any psychopathy.

In regards to the reasons behind the actions, the subject spoke of gratification prior to the assault linked to predatory behavior, in the idea of choosing a victim and having her under his power. The sexual excitement was more mental than physical in light of the fact that he

never orgasmed with any of the women. He also expressed disappointment in the one sexual encounter he had.

What is unique about this case is that although responding to the motivational profile of 'Power Rape' by Groth, the subject used power fantasies brought on through the assaults for arousing himself and consummating the sexual act later at home with his wife. The hypothesis, which finds an almost concrete demonstration in this case, is that in this type of rapist, sexual abuse is none other than violence perpetrated through a sexual act. The man 'uses' this violence – which serves to charge him psychologically and give him power – to sustain a sexual rapport with other women at a later time. He looks for power situations which can compensate his belief of impotence, and then uses them as 'realistic fantasies' which are utilized while having sex with his wife or during masturbation.

The feelings of impotence and inadequacy were in this case fed not only by a self belief that he had a small penis, but also through physical problems; since the age of fifteen he was an insulin dependent diabetic who recently had been suffering erectile dysfunctions.

The underpinning problems were connected to a need to reinforce a weak "Self", and a fear regarding sexual intercourse when the subject's background became apparent; at the age of 20 he had not yet had any sexual experiences. Once enrolled in the Police Force, he assaulted a young woman with his service pistol, attempting to rape her but without successful penetration, despite the desire to do so. Once identified, he confessed to the charges and was convicted. However, once released he married and lived a normal life.

**Serial Sexual Assault, Sexual Fantasies, Power Fantasies**

## I22 Shared Religious Psychotic Disorder in Three: Living With the Corpses of Two Dead Sisters

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After attending this presentation, attendees will gain an understanding of some of the psychological impairment aspects of an elderly woman living with the corpses of her sisters for a two-year period. The goal of this presentation is to shed light on the often under-reported and rarely encountered phenomenon of "shared psychotic disorder" and their associated contributing factors in this case of ritualism, segregation, and family traditions.

This presentation will impact the forensic community by offering an extraordinary story of a woman who lived in such a situation as a result of being in a state of religious paranoid delusion.

On August 10, 2007, police entered an isolated villa in the open countryside of Apulia, Italy, a few kilometers from the town center, where a 70-year-old woman was discovered. She was found to be in a state of confusion and poor hygienic condition. The mummified cadavers of her two dead sisters were found, along with the carcasses of five cats and three dogs. The three sisters, who had never married, lived in the small house for many years where they held prayer groups and remained in isolation for eight years prior to the death of the first sister.

A farmer periodically provided for their general needs (food and supplies) which he placed at their door, without ever going inside the house. The results of the medico-legal investigations completed showed that the deaths had occurred 18 and 15 months before the crime scene investigation. The cause of death for one of the sisters (73-year-old) was the result of a left femoral fracture which had not received necessary medical attention. In the case of the other sister, who was 78-year-old at the time of death, no signs of traumatic lesions or toxicological evidence was found.

The survivor, who was in a state of malnutrition, underwent psychiatric evaluation which excluded any detectable psychological pathology. After reading the diaries found at the investigation scene, and hearing information furnished by the surviving woman, it was possible to reconstruct the entire story. Immersed in an intensely religious life, and looking for signs from God, the sisters' extreme isolation triggered a collective delusion of a religious nature, replete with daily "divine confirmations." The deaths of the sisters were experienced with a sense of disbelief, but the surviving sister had not given up hope. She imagined that God "would do something", and therefore, continued to pray for hours on end, waiting for "divine" signs.

Living in this way with the cadavers of her sisters, and over time, neglecting to feed the dogs and cats, they eventually died of starvation. The woman prayed in a makeshift chapel which was nothing more than an old garage, where in the past the sisters had also held prayer groups. It seemed that there was also an altar in the "chapel" which had been consecrated by a local priest.

After careful analysis of the entire chain of events in question, the diagnosis of "follia condivisa a tre" (madness shared by 3) was made.

Shared psychotic disorder was first described in 1877 as *folie à deux*. It is a rare disorder shared by two or more people with close emotional ties. Cases involving three or more people are very uncommon. Information regarding the incidence and prevalence of shared psychotic disorder is lacking, as the literature consists entirely of case reports. Among siblings, the disorder is more common in sisters than in brothers. Almost all cases involve members of a single family.

**Religious Paranoid Delirium, Neglect, Death Investigation**

## J1    Validation of an Automated Handwriting-Derived Biometric Identification System (FLASH ID) on English and Arabic Writings

*Mark A. Walch, MBA\*, The Gannon Technologies Group, 1000 North Payne Street, Alexandria, VA 22134*

The goal of this presentation is to illustrate an automated technique for handwriting derived biometric identification that transcends languages and scripts. The biometric power of handwriting is embedded in graphemes - compact graphical forms - that exist in all languages.

This presentation will impact the forensic science community by empowering forensic document examiners to new frontiers—by extending their reach into new languages - that have previously been perceived as barriers.

This presentation will provide updated information regarding FLASH ID; a highly effective means for automatic handwriting derived biometric identification. The "FLASH ID" software package was developed with extensive guidance and technical input provided by forensic scientists from the Federal Bureau of Investigation Laboratory.

A specific aspect of FLASH ID's functionality will be presented in the form of its "language independence." Since FLASH ID decomposes writings into individual "graphemes" which transcend individual languages, it can perform writer identification in multiple languages either concurrently or independently. This ability to extend Forensic Document Examination across multiple languages represents a significant breakthrough over current practices which tend to be language specific.

The audience will learn that the biometric power of handwriting does not rest with individual characters or words, but takes the form of parts of characters and character connectors. Handwriting biometric power also does not rely on any particular script, but rather, it crosses both languages and scripts. The mathematical underpinnings of FLASH ID are based on Graph Theory and FLASH ID works by quantifying graphical features, available within an individual's writing, into a "loss less" data structure that preserves the topology and geometry of the original writing. The methods for creating this data structure as well as the actual structure are "language agnostic." That is, the data structure is built from graphemes—graphical forms—rather than actual characters from a particular language. This data structure captures both the topology and hundreds of detailed physical measurements from written forms. Using this graph-based format, FLASH ID employs statistical methods to distill the topological and physical features into a "biometric kernel." The Biometric Kernel captures the essence of the repertoire of physical forms used by a particular individual to write in a particular language.

Specific experiments to validate the language independent capabilities of FLASH ID when applied to the identification writers in a language other than English will be discussed. For purposes of this presentation, Arabic is the chosen language. Topics to be presented include: similarities and dissimilarities in the graphical composition of language; empirical evaluations of grapheme distributions between languages; biometric writer identification performance on collections of English, Arabic, and mixed commingled documents; and the feasibility of capturing class characteristics of languages. The presentation will culminate in a live demonstration showing FLASH ID applied to both English and Arabic documents in the same collection.

A key point to be made to the audience is FLASH ID represents a new approach toward handwriting examination that will empower forensic document examiners to handle documents in multiple languages. The core message will be rooted in two important aspects of the technology used to build FLASH ID. First, FLASH ID represents a totally automated process for extracting biometric data from handwritten documents regardless the source language, analyzing these data using established statistical methods and matching documents based on similarity of the captured writing to known writing. Second, the technology underlying FLASH ID's language independence has been demonstrated to function in different languages with completely different scripts.

As a residual biometric that can link individuals to documents they have written, handwriting provides an important data source for both law enforcement and intelligence purposes. FLASH ID provides the forensic science community with a tool that harnesses the power of automation to make current practices more efficient and effective. By transcending language, the impact of FLASH ID is that it will empower forensic document examiners to new frontiers—by extending their reach into new languages—that have previously been perceived as barriers.

**Handwriting, Biometric, Arabic**

## J2    Evaluation of the Language-Independent Process in the FLASH ID System for Handwriting Identification

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After attending this presentation, attendees will be exposed to some of the "inner workings" of the FLASH ID system. This will show that the FLASH ID system is not attempting to replicate the actions of a document examiner, but will present the examiner with a powerful new tool to assist in the identification of questioned documents. By understanding better the methods behind the language - independent mode, the attendees will appreciate its potential for assisting document examiners. Attendees will be exposed to a study demonstrating the effectiveness of the language-independent mode in identifying the writers of questioned documents.

This presentation will impact the forensic science community by informing them about a system that can assist forensic document examiners to identify the writer of a questioned document. This presentation will present information about the statistical methods behind the language-independent mode of operation and give results that show the excellent performance of the system.

FLASH ID was introduced to the forensics community at AAFS 2008. FLASH ID is a totally automated system for handwriting identification that can operate in either a character-based or language-independent mode. At the 2008 AAFS meeting, a quantification of handwriting and its application to handwriting identification were described. An evaluation of how the FLASH ID system performed using the character-based mode was presented. That mode is based on segmenting handwriting into alphabetic characters, which are then associated with graph structures. In the language-independent mode, the

alphabetic dependency is removed through a language-independent segmentation algorithm for handwriting. The resulting segments do not necessarily correspond to any alphabetic characters. This presentation demonstrates the language-independent segmentation and gives performance results when it is the basis for identifying the writer of a questioned document. A major advantage of language-independent segmentation is that it does not require character recognition in order to segment handwriting. The language-independent technology has been implemented in FLASH ID for two years.

This presentation will contain a discussion of the language-independent segmentation algorithm and examples of its implementation. The segments of handwriting generated by the algorithm are associated with graphic entities that provide a topological classification and geometric features (just as for the character-based mode). Shape codes are simple features that are used to define sub-categories for each class of graph. The combination of a graphic class and a shape code category is called a grapheme. Handwriting is then modeled at the grapheme level. That is, each writer with known writings is characterized within each grapheme that occurs with some frequency in the known writings. A questioned document is broken down into graphemes and each writer in the database of known writers receives a score for each occurrence of a grapheme in the questioned document. Scores for all occurrences of graphemes in the questioned document are summed for writers. These total scores are used to rank writers by likely writer-ship of the questioned document. The total scores and the associated ranks are displayed by FLASH ID for evaluation by forensic document examiners. This presentation will illustrate the excellent performance results for the language-independent process in multi-language applications.

**Forensic Document Examination, Automated Handwriting Identification, Statistics**

## J3    Handwriting Individuality:  Probability Models, Subsampling Routines, and Implications

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After attending this presentation, attendees will understand the relationships between forensic handwriting individuality and random match probability, the use of subsampling to estimate the random match probability (RMP), and the interconnection between the strength of handwriting evidence and the size of the document.

This presentation will impact the forensic science community by providing bounds on the RMP that are based on the size of the questioned document, as well as quantifying the effect of the sample size of the document on the strength of handwriting evidence.

Forensic handwriting individuality refers to the proposition that each individual in a population has a unique writing profile. An empirical study cannot validate this proposition due to the impossibility of observing sample documents written by each person in a relevant population. However, the proposition that writing profiles are unique is one of the key premises underlying forensic handwriting comparisons.

In Saunders et al. (2008),[1] the relationship between forensic handwriting individuality, biometric individuality, and random match probabilities using a convenience sample of documents collected from 100 individuals was explored. The random match probability (RMP) of interest in handwriting analysis is the chance of randomly selecting two individuals from some relevant population and then randomly selecting a writing sample from each individual that are declared to "match" by a specific biometric matcher. A complementary probability, the random non-match probability (RNMP), is the chance of randomly selecting an individual and then randomly selecting two writing samples from the selected individual's body of handwriting that fail to "match" using the chosen biometric matcher. The RMP and the RNMP both depend upon the biometric matcher used and the sizes of the documents compared, as well as the relevant population from which individuals are selected.

Generally speaking, the RMP decreases as the writing sample size increases, with the theoretical minimum being the biometric individuality. The biometric individuality (of a population with respect to a comparison methodology) is defined as the probability that two (different) randomly selected writers from the population have indistinguishable writing profiles (with respect to the comparison methodology being used). Intuitively, two writing profiles being indistinguishable means that one concludes that the handwriting of two writers looks the "same" after observing their entire body of handwriting. The biometric individuality is bounded below by the handwriting individuality of the population. Therefore, a bound on the RMP will bound the biometric individuality and the handwriting individuality of a given population.

Using the subsampling routines presented in Saunders et al. (2008),[2] the behavior of the RMP is consistently estimated through an empirical subsampling routine. Research to investigate how subsampling from available writing samples in a data set can be used to investigate the dependency of the RMP and RNMP on the sizes of the writing samples being compared. The consistency of the subsampling estimates is dependent only on the number of writers. Based on an Federal Bureau of Investigation database of about 500 writers with approximately five writing samples per writer (the "Federal Bureau of Investigation500" data), results of the modeling will be presented and review the corresponding implications to a handwriting individuality study.

The subsampling routines provide a natural way to estimate the strength of handwriting evidence as a function of the subject's writing sample size. When evaluating the strength of evidence, there are two competing hypotheses of interest. The first hypothesis is that the subject is the writer of the questioned document, and the second hypothesis is that that random person (not the subject) is the writer of the questioned document. To evaluate the strength of the evidence, a likelihood ratio is used which compares two probabilities:

The probability of a match between the questioned document and a writing sample of a given size obtained from the subject.

The probability of a match between the questioned document and a writing sample (of the same given sample size) obtained from a randomly selected individual from an appropriate population.

The results of a short empirical study from the Federal Bureau of Investigation500 data that models the strength of evidence as a function of the writing sample size is presented.

The proposed estimation methods and the associated conclusions concerning handwriting uniqueness will be illustrated and compared using the handwriting biometric identifiers.

**References:**
1.    Saunders, et al., "A Comparison Between Biometric and Forensic Handwriting Individuality," *Journal of Forensic Science* (submitted 2008).
2.    Saunders, et al., "Modeling the Relationship Between Random Match/No-Match Probabilities and the Sizes of Writing Samples," *Seventh International Conference on Forensic Inference and Statistics, Lausanne, Switzerland (2008).*

**Handwriting Individuality, Random Match Probability, Subsampling**

## J4 Validation Testing for FLASH ID on the Chaski Writer Sample Database

*Carole E. Chaski, PhD, Institute for Linguistic Evidence, 25100 Trinity Drive, Georgetown, DE 19947; and Mark A. Walch, MBA\*, The Gannon Technologies Group, 1000 North Payne Street, Alexandria, VA 22134*

After attending this presentation, attendees will understand how to evaluate validation tests in general and specific validation testing results of testing FLASH ID (Walch et al, 2008) on a forensic handwriting database (Chaski 1997, 2001). Validation testing is a key component of developing new technologies in forensic science as well as a legal qualification for admissible scientific and technical evidence.

This presentation will impact the forensic science community by meeting a major requirement of the *Daubert* challenge to handwriting identification, i.e., demonstrating that FLASH ID has been validated on a forensically feasible database of known handwriting samples with a low error rate such that these results provide evidence of how well FLASH ID can perform on accurately classifying handwriting in actual cases.

Validation testing is a four step process. First, validation testing requires a database of known samples whose characteristics mirror as closely as possible the actual samples found in actual cases. Second, validation testing requires an objective method being repeated on all the samples. Third, validation testing requires a cross-validation scheme which tests the accuracy of the method for correctly identifying the known writers. Finally, validation testing requires a calculation of an error rate based on the cross-validated accuracy.

For the validation testing of handwriting identification methods, the first step of finding a database of known samples whose characteristics mirror actual data has been challenging. Some available handwriting databases do not contain known samples. It is impossible to test a method's ability to identify if there is any confusion at all about the identity of any of the samples. Some available handwriting databases do not contain samples whose characteristics mirror actual samples. Spontaneous text offers the best way to predict actual performance under actual conditions involving forensic document examination. Spontaneous writing is also important linguistically since it will mirror the actual phonotactics (frequency of letters, letter-positions, and letter-combinations) of the language, whereas copied handwriting only shows the artificial frequencies of the model. The available models do not accurately capture English phonotactics.

The Chaski Writer Sample Database (Chaski 1997, 2001) provides spontaneous handwriting from known writers. The database has previously been used to validate linguistic methods for determining authorship by research teams in the United States, Canada and Switzerland. This presentation describes the first use of the database to validate computational methods for identifying handwriting. The database contains approximately 175 known writers whose demographic information is known. This demographic information includes the age, race, sex, educational level, dialectal information, graphic type (cursive, print, mixed), and legibility. Each author has authored at least three texts on topics which are sociolinguistically determined to evoke different communicative functions and emotive states (e.g., describe a personal trauma or frightening experience, describe career goals). Some authors are not useful to this study since their documents are typed or computer-generated.

Each writer wrote on unlined paper, selected his/her own writing instruments and wrote at his/her leisure. Each writer produced as much on each topic as she/he desired. Thus, the Chaski Writer Sample Database mirrors the characteristics of actual forensic data because in actual cases, for the knowns, the writing instruments, the amount of handwriting, the communicative functions of the texts can vary, and the writers are writing freely without copying a standard text. For 100 writers, at least three writing samples and up to eight writing samples were extracted. The writing samples vary in length from quarter-page to full page.

Each writing sample was processed through FLASH ID using a leave-one-out cross-validation scheme. Further, varying amounts of text for each writer, in 150 character increments, were processed. Thus, this presentation reports the results of validation testing FLASH ID for writer identification in general and under specific conditions of text length, instruments, and graphic type.

**Handwriting Identification, Validation Testing for *Daubert*, Forensic Database**

## J5 Individuality of Handwriting: A Twins Study

*Sargur Srihari, PhD\*, University at Buffalo, 520 Lee Entrance, Suite 202 UB Commons, CEDAR, University at Buffalo, Buffalo, NY 14228*

After attending this presentation, attendees will gain an understanding of: (1) the extent of individuality of handwriting as measured by the comparison of the handwriting of a significant number of twins, and (2) performance of automated methods in handwriting verification in comparison with that of lay persons and professional examiners.

The presentation will impact the forensic science community by providing a measure of individuality of handwriting and error rates.

Since handwriting is influenced by physiology, training, and other behavioral factors, a study of the handwriting of twins can shed light on the individuality of handwriting.

A study of writer verification for twins and non-twins is presented using an automatic handwriting verification system. The system performs the task through a statistical model of similarities between a set of features extracted from each of the handwriting samples. Handwriting samples provided by 206 pairs of twins as well as by 206 pairs of non-twins were used in the study. For twins, the experiment consisted of 1,236 tests (including 824 different content and 412 same content pairs), where the task is to determine whether two half-page documents were written by the same individual. The results show that the handwriting of twins tend to be more similar than that of non-twins: the verification error rate of twins is about 13% while non-twins' error rate is around 4%. Error rates with identical twins is higher than that of fraternal twins. Error rates in all cases can be arbitrarily reduced by rejecting (not making a decision) on borderline cases. System performance is seen to lie in-between that of lay persons and professional forensic document examiners.

**Twins' Handwriting, Automated Writer Verification, Human and Machine Performance**

## J6 How Does a Document Examiner Differentiate Authors?

*Yoko Seki, MA\*, National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa-shi, Chiba-ken, 277-0882, JAPAN*

After attending this presentation, attendees will understand the difference in the pattern recognition between human and the computer based superposition. Attendees also will understand the availability of the human pattern recognition to the handwriting identification.

This presentation will impact the forensic science community by demonstrating the superiority of the human pattern recognition to the computer based superposition in the handwriting identification under a certain condition.

A document examiner examines handwriting mainly by a qualitative method based on his/her knowledge and experiences. The qualitative examination, compared with the quantitative examination,

possesses less objectivity and is believed to be less reliable. However, an examiner's opinion is, in fact, highly reliable. This is because the examiner has much knowledge about the handwriting and chooses the strategy and variables that are most appropriate to his/her case. So, an analysis of the strategies and variables an examiner uses and the quantification of them will contribute to the establishment of the objectivity in the examination.

Six subjects wrote 24 kinds of Japanese characters six times in square style. Six out of 24 kinds were Hiragana characters and 18 out of 24 kinds were Kanji characters. Each kind of character had 36 samples (6 subjects x 6 times) and all the samples collected were eight hundred and sixty-four characters, that is, 24 kinds of characters x six subjects x six times. Then, 36 samples of the same character were classified into six groups following the procedures below: (1) Cluster analysis: Each handwritten sample was measured its x-y coordinates at 21 measuring points defined beforehand. Coordinates were standardized as for the origin and the size. Cluster analysis was done using standardized coordinates. Cluster analysis finished at the stage where clusters were merged into 6, (2) Classification by the visual examination: This was similar to a case work. A document examiner observed 36 samples and classified them into six groups according to the similarity of the samples, and (3) Cluster analysis based on the variables used for the visual examination: The examiner was interviewed about the variables used for the classification after the trial. Coordinates of characters were measured and calculated based on the variables used by the examiner and then cluster analysis was done.

Correct classification ratios of the three classifications methods were calculated and compared. Correct classification was defined as follows: A cluster was defined to be equal to the subject whose samples were contained in the cluster most. If a cluster had four samples of the subject No.1, one sample of the subject No.2 and one sample of the subject No.3, the cluster was defined as subject No.1's. After labeling each cluster to the subject, correct classification ratio was calculated. Correct classification ratio was defined as the ratio of correct subject's samples to the whole samples (=36 samples).

Classification by the visual examination showed the highest correct classification ratio in the three methods. Correct classification ratio was higher in the cluster analysis using coordinates than the cluster analysis using the observation variables. Variables used for the visual examination were the relationship between the components in space and the size. Thinking that the number of the variables is much smaller in variables used for the visual examination than the coordinates (=42 variables), human recognition is more effective than pattern matching using the superposition of the patterns under the condition that the task was the classification of small number of data into several groups. These suggest that the human recognizes a pattern roughly and focuses the relationship between the components, different from the local pattern matching.

**Handwriting Identification, Cluster Analysis, Visual Examination**

## J7 A Novel Method for the Examination and Characterization of Documents Printed With Inkjet Printers

*Rebecca L. Schuler, BSc\*, ChemImage Corporation, 7301 Penn Avenue, Pittsburgh, PA 15208; and Cara A. Plese, LaRoche College, 9000 Babcock Boulevard, Pittsburgh, PA 15237*

After attending this presentation, attendees will understand the basic principles of hyperspectral imaging and how hyper spectral imaging can be a useful method for inkjet printer ink analysis.

This presentation will impact the forensic science community by introducing a new, nondestructive method for characterizing different brands of inkjet printer inks.

The pricing and widespread market availability of ink-based home/office machine systems (printers, copiers, fax machines) has yielded an increase in the submission of printer ink-based material evidence to the Forensic Document Examiner (FDE). For this type of evidence, various types of examinations occur, including paper analysis, physical examinations, and chromatographic methods. Unfortunately, chromatographic methods, including Thin-Layer Chromatography (TLC), are destructive to the evidence and physical examinations do not usually provide enough information about the sample. Due to the increasing number of submissions of printer ink-based documents, the ability to categorize, discriminate, and/or identify colored inks from different manufacturers, using a nondestructive method is important to the FDE.

In this study, the feasibility of hyperspectral imaging as a technique for examining inkjet printer documents is explored. Hyperspectral imaging combines digital imaging technology with conventional spectroscopy for evidence analysis. It provides high spatial resolution, high image definition, and full spectrum analysis. In operation, digital images of the sample are recorded as a function of wavelength through the use of an electro-optic imaging spectrometer, generating a fully resolved spectrum for each pixel location in the multi-frame image. The combined spatial and spectral information reveals subtle features of a material that, often, cannot be observed using traditional imaging techniques. Hyperspectral imaging is a validated technique that is becoming a commonly utilized technology for the analysis of other types of questioned documents (travel documents, pen inks, etc).

Various inkjet printers were selected to print an assortment of pictures. The pictures were examined using hyperspectral imaging technology. It was found that the cyan, yellow, and magenta components of each type of inkjet printer brand have their own unique spectroscopic responses. The results demonstrate how hyperspectral imaging can be used as a nondestructive method for connecting different printer samples to a common manufacturer or ink formulation.

**Hyperspectral Imaging, Inkjet Printer, Questioned Documents**

## J8 Study on Hangul and Handwriting Identification Methods in Hangul

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After attending this presentation, attendees will understand how handwriting is a different position of characters with individuals, and how handwriting characteristics can be identified by comparing external shape.

This presentation will impact the forensic science community by demonstrating the alteration of individual handwriting, and how handwriting changes according to position of consonant.

Hangul is the name of the Korean alphabet that was invented in 1443 by several scholars at the order of the Great King Sejong of the Josun Dynasty. Hangul is a phonetic alphabet, not an ideograph as some may think it is. Hangul has 24 basic characters 14 basic consonantal characters and 10 basic vowel characters. Each Hangul syllable is composed of a syllable-initial consonantal character, a syllable-peak vowel character, and optionally a syllable-final consonantal character. In large part this is because the individual characters are not concatenated linearly to form words as Westerners are used to, but are grouped together to form two-dimensional representations of syllables, which are then arranged linearly to form words. According to the shape of the vowel and the existence of the bottom consonants, the type of Hangul

character can be divided into six categories. The type of Hangul character can be divided into six categories. Six types are divided into horizontal character, vertical character and mixing character to use handwriting identification. In case of the alphanumeric characters, it is well known that some special conventions in order to differentiate intrinsically similar characters are used. For example, Ø (/(slash) and 0 (zero)) in order to write 0 (zero) are used so that it can easily be separated from o (oh). The number of all legal Korean characters is 11,172 as described in Section 2. Even the number of the characters for the daily use is 2,350. Therefore, basic analysis is needed about the stroke and exact structure of Hangul. Hangul handwriting is composed by consonant and vowels combining horizontal character, vertical character and mixing character. Because handwriting is different position of character with individual, handwriting characteristic can be identified by comparing external shape. Consonant constituted more than 2-stroke characters is different stoke method from writer. Particularly, the characteristic of ' ' (mi-eum), ' ' (bi-eup), ' ' (pi-eup), is observed with different general stoke method. The stroke angle often appears on vowels in handwriting characteristic. Stroke angle is a compared factor of handwriting identification in direction of stroke, finish method of curved parts. When words and phrases are composed, special change of handwriting and combining between handwriting are factors of handwriting identification.

This experiment was analyzed through various method followed by consonant position of the same person handwriting. First, handwriting characters was to classified by three types of classification systems and confirmed through handwriting physical character. Individual characters were analyzed statistically looking at spacing between words, variation in spacing between lines, variation in spacing between characters, number of angularity, number of corrections, number of tremor, etc.

In this study, the alteration of individual handwriting and handwriting change according to position of consonant is a foundation of objective estimation in handwriting identification.

**Hangul, Handwriting Identification, the Alteration of Individual Handwriting**

# J9    Thermal Imaging of Obliterated Writing

*Diane K. Williams, PhD, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135; and Kerri L. Moloughney, BS\*, Oak Ridge Institute of Science Education, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will understand the use of thermal imaging to view obliterated writing evidence.

This presentation will impact the forensic science community by increasing understanding in the visualization of obliterated writings.

The use of thermal imaging techniques for the non-destructive examination of obliterated writings could be of significant value for questioned document analyses. The commonly used Video Spectral Comparator (VSC) is able to decipher many questioned writings, but has been found to have limitations, most notably in its inability to visualize "true black" inks. A "true black" ink is non-fluorescent and non-reflective and has traditionally posed a challenge for questioned document examiners. Additionally, the VSC is limited to deciphering obliterations in which the inks have significantly different spectral properties. As a possible alternative method, research has been performed to determine the feasibility of visible/near infrared hyperspectral imaging in reading obliterations[1]. This technique has been found to be very effective, but has some limitations as well. In some cases, pencil/pen obliterations were not able to be read, due to the lack of a significant spectral signature of the pencil writings.

Thermal imaging is an alternative tool that can aid in the visualization of obliterated writings. Infrared thermography utilizes the amount of infrared light emitted by objects and chemicals to determine their approximate temperature and provide contrast based on the differences. When heated, the unique emissivities of inks cause different amounts of radiation to be emitted, enabling the image to be based on temperature and chemical differences rather than wavelengths. The early results of this proof-of-concept study indicated that several operating parameters can be adjusted to yield optimum results. Therefore, all of the samples that were collected for the initial study have been reanalyzed. To ensure the reproducibility of the results, two identical cameras were used to collect the thermal images.

To validate the technique, multiple blind samples of obliterated writings were analyzed. All samples were written in #1, 2, or 3 pencil, black pen ink, true black ink, black gel pen ink, or were typed on paper. The implements used to obliterate the writing were black ink, true black ink, black gel pen ink, marker, crayon, and white out. The obliterated writing samples included printing in all capital letters, cursive writings, a combination of print and cursive writings as well as the type written samples. Most samples were heated so that the emissivities of the inks could induce a thermal contrast. This technique enables the user to visually decipher between the inks present, allowing the original writing to be read. A few samples could be discerned without heating but the vast majority did require heating. Several heat sources were used but the most commonly used source was a standard 120W light bulb. Many writing implement combinations are easily and quickly visualized; including any pencil, gel pen, or printer ink writings obliterated by various other implements. These results indicate that thermal imaging is a valuable tool in the analysis of obliterated writings, especially when used in conjunction with hyperspectral imaging.

**Reference:**

1.    Ayub, H., Williams, D.K., "The Role of Hyperspectral Imaging in the Visualization of Obliterated Writings", American Physical Society, Baltimore, Maryland, March 2006, abstract no. R9.6

**Obliterated Writing, Thermal Imaging, Ink Analysis**

# J10    Infrared Examination of Altered Ink Handwriting Using Hand-Held Digital Infrared Scope

*John Z. Wang, PhD\*, California State University-Long Beach, 1250 Bellflower Boulevard, Long Beach, CA 90860*

After attending this presentation, attendees will understand basic principles of infrared examination of altered ink handwriting, functions of a new hand-held digital scope, and applications of infrared capacities with the new device.

This presentation will impact the forensic science community by providing a new technical direction for the questioned document examination community on the topic of altered ink handwriting identification.

Traditional devices such as Thin-Layer Chromatography (TLC) and Gas Chromatography/Mass Spectroscopy (GCMS) have been used to examine altered ink handwriting, but they are lab-based instruments and related examinations should be considered to be destructive or semi-destructive. Quick Scan Infrared Spectrometer (QSIRS) can provide easy operation with quick data collection and does not require purge of moisture and $CO_2$ prior to use, allowing easy and fast sample analysis. However, the equipment is very expensive.

A case involved with altered ink handwriting is introduced to illustrate the new device: Hand-Held Digital Infrared Scope. The device is inexpensive (approximately $1,200), portable (hand-held), laptop connection (USB 2.0 port), digital (image sensor 1/3" CMOS & 2.3 microns), three capture functions (still image, movie, and time lapse), two infrared wavelengths (850 nm & 760 nm), continuous magnification (40x ~ 140x), and digital measurement (on screen). The digital

measurement components include: (1) multiple geometrical measurements (line, triangle, circle, arch, and rectangular), and (2) added on capabilities (labels, markers, time stamp, and drawing). It is suggested that this new device is able to introduce two new examination dimensions: portable infrared examination and digital measurement for the future.

**Digital Measurement, Infrared Scope, Altered Ink Handwriting**

## J11 Can Dynamic Features be Used to Discriminate Between Forged and Disguised Signatures?

*Linton Mohammed, MFS\*, San Diego County Sheriff, Crime Lab, 5255 Mount Etna Road, San Diego, CA 92117; Bryan Found, PhD, La Trobe University, Bundoora Campus, Melbourne, 3086, AUSTRALIA; Michael Caligiuri, PhD, University of California, San Diego, 3350 La Jolla Village Drive, San Diego, CA 92161; and Doug Rogers, PhD, La Trobe University, Bundoora Campus, Melbourne, 3086, AUSTRALIA*

After attending this presentation, attendees will become aware of dynamic features that may help them to improve their ability to discriminate between forged and disguised signatures.

This presentation will impact the forensic science community by increasing the objectivity of handwriting examinations.

The examination and comparison of signatures is a task routinely carried out by Forensic Handwriting Examiners (FHEs). Empirical blinded studies of FHEs' skills have shown that they are proficient in discriminating between genuine and forged (simulated) signatures. In real-case scenarios; however, FHEs must also consider the possibility that the true (exemplar) writer may have produced a disguise signature in order to later claim that the signature was forged. In these cases, the disguise strategy adopted by the signer must result in a signature displaying a pictorial similarity to the writer's real signature style. This is to maximize the possibility that it would pass a cursory inspection at a transaction point by any person who might have access to a specimen signature. It is accepted that that writers' employing disguise behavior often incorporate elements or features that they can point at later as evidence of forgery (for example changes to the design of initial characters in the signature form). Some disguise behaviors can be more complex and can result in altered features that are typically associated with forgery behavior. Recently reported large scale signature validation trial results indicate that FHEs, when treated as a group, perform poorly on tasks involving the discrimination between forged and deliberately disguised signatures. It is clear that additional research effort is required to focus on the features that might best predictor whether a questioned signature is disguised or forged.

In this study, 30 writers were asked to forge the signatures of three specimen signature providers (the "model" writers). The signature style of each of the three models was different. One was a text-based signature (where all the allographs were legible). The second signature was a mixed style (where two or more, but not all of the allographs were legible) and the third was stylized (where none of the allographs were legible). The model signature writers each produced 20 signatures (ten genuine, five disguised, and five signatures disguised to look like forgeries). All signatures were written on a digitizer pad (sampling at 200Hz with 0.0005 cm resolution) which measured dynamic features such as velocity, duration, jerk, size, and pen pressure.

The forgers each provided ten genuine signatures and fifteen forgeries of each of the three model signatures. Each forger practiced three times on plain paper, before producing the forgeries on the digitizer. This resulted in a database of 1,350 forgeries. The dynamic data was analyzed statistically and the features of the genuine, disguised, and forged signatures were inter-compared. The data from the first five

forgeries and the last five forgeries of each writer was statistically compared to determine if there was any learning process. A Likert survey was conducted of the forgers to determine qualitatively which if the three signature styles they found most difficulty to forge. The analysis should provide empirical evidence of any predictors that might assist a FHE to improve the reliability of discriminating between disguised and forged signatures.

**Forensic Document Examination, Signatures, Forged, Disguised**

## J12 Forensic Document Examiners' Expertise in the Examination of Text-Based Signatures

*Derek L. Hammond, BA\*, U.S. Army, Criminal Investigations Lab, 4930 North 31st Street, Forest Park, GA 30297-5205*

The goal of this presentation is to provide attendees with an overview of the performance results stemming from Forensic Document Examiner (FDE) participation in a skill-based test designed to assess the expertise (e.g., skill) of a group of FDEs tasked with rendering opinions regarding the authorship of genuine, disguised and simulated text - based signatures.

This presentation will impact the forensic science community, and in particular, the forensic document examination community, as data addressing FDE reliability regarding the examination of genuine, disguised and simulated text-based signatures has not previously been reported.

A group of FDEs (N=11; 5 individual FDEs and 3 peer groups consisting of 2 FDEs per group) were tasked with comparing a set of known specimen signatures (N=18) written by a single writer to a set of questioned signatures (N=60). The questioned signatures were comprised of 9 genuine signatures written by the specimen writer using their normal signature style; 6 disguised signatures written by the specimen writer; and 45 simulated signatures written by a group of 8 lay-forgers. Participants were provided with 4" x 6" commercially printed images of the previously scanned (600 ppi) original questioned and known signature specimens.

The participant group, which did not include any laypersons, are all FDEs employed in local (N=2), state (N=1), federal (N=6), or private (N=1) forensic laboratories. The experience levels of this group varied: 1-5 years (N=3), 6-10 years (N=1), 11-15 years (N=2), 16-20 (N=1), 21-25 years (N=3), and >26 years (N=1).

A total of 480 authorship opinions were expressed by the FDE group. In terms of called opinions only (excludes all inconclusive opinions), 8 misleading opinions (3.2% of called opinions) and 242 correct opinions (96.8% of called opinions) were expressed by the group. Overall, 230 of the 480 opinions expressed were inconclusive (47.9%), 242/480 opinions (50.4%) were correct and 8/480 opinions (1.7%) were misleading (i.e., erroneous identification/elimination). The questioned "genuine" signatures did not attract any misleading opinions. One (1) of the "disguised" signatures produced an erroneous finding as did 7 of the "simulated" signatures. Although eight "forgers" were utilized to produce the 45 simulated signature specimens, only one "forger" succeeded in producing simulations (N=7) of the specimen's writer normal signature resulting in misleading opinions. All of the misleading opinions expressed (N=8) were generated by a single FDE. Furthermore, all of the misleading opinions were "qualified" (i.e., less than definitive). The remaining seven answer booklets did not contain any misleading opinions.

Historically, FDEs have demonstrated a high correct called rate and low inconclusive and/or misleading rates associated with determining the authorship of "genuine" signatures. In contrast, lower correct called rates and higher inconclusive and higher misleading rates and have been associated with authorship opinions stemming from FDEs examination

of non-natural writing types (e.g., disguised and/or simulated signatures). In this regard, the data reported from this study appears to be consistent with previously published research addressing FDE expertise for similar tasks (i.e., signature comparisons).

**Reliability, Error Rates, Text-Based Signatures**

## J13    Charred Documents

*Gregg M. Mokrzycki, MFS\*, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135; and Gabriel D. Watts, BA, Questioned Documents Unit, Room 2160, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will develop an understanding of the fragile nature of charred document evidence and will learn techniques for recovery and maximizing the potential evidentiary value of it.

This presentation will impact the method for collecting charred documents and will be useful to questioned documents examiners, crime scene personnel, and other forensic scientists that encounter charred evidence.

This session will be a "cradle to grave" demonstration showing the best methods of collection, information recovery, and preservation of charred documents. Attendees will get hands-on exposure to charred documentary evidence, and will test methods for collecting, photographing, and preserving the evidence.

**Charred, Documents, Crime**

## J14    Looking at How to Differentiate Measurements Used to Test Printing on Documents

*George Virgin, MS\*, FDL, 8000 Westpark Drive, Suite 200, Mclean, Virginia 22102; and Walter F. Rowe, PhD, Department of Forensic Science, George Washington University, 2036 H Street, Washington, DC 20052*

After attending this presentation, attendees will have learned about a semi-automated method used to test the similarity and differences of printing on documents.

This presentation will impact the forensic science community by familiarizing the forensic community with the potential of combining these methods with automated print quality testing.

Forensic document examiners can utilize automated print quality testing instruments to authenticate the printing on a document; however, in order to utilize automated print quality testing a scientist needs to instruct the instrument with which measurements to use to test the document. Scientists may select measurements that are likely to be most important to differentiate between a questioned and known document. Depending on the document being tested there can be many measurements to select from. Scientists may not be sure that the measurement they chose will differentiate documents until after they do further testing using an automated print quality testing instrument. It can take a lot of data results to select the best measurements to use. In this study a desktop scanner and computer software were used to select from a group of measurements, the measurements to test that were most differentiating between the printing on a questioned and known document. Mathematical tests (k-means clustering, hierarchical clustering, principal component analysis and discriminant function analysis) were performed and differences and similarities between the questioned and known documents were observed and graphed. More testing can be done and there is potential to apply this method to different types of print quality measurements and different types of documents. Results obtained using a scanner and software could be combined with automated print quality testing instruments to help select the most differentiating measurements. This method could also be used as a quick assessment of certain similarities and differences of printed characters and artwork on a questioned and known document. Current testing in this field using statistical methods will also be covered.

**Questioned Documents, Printing, Desktop Scanner**

## J15    Examination of Blue Gel Inks

*Walter F. Rowe, PhD\*, and Stephanie Moore, MFS, George Washington University, Department of Forensic Sciences, 2036 H Street Northwest, Washington, DC 20052*

After attending this presentation, attendees will understand the composition of blue gel inks and become aware of the best combination of analytical methods to be used to identify the brand of blue gel pen used to create a handwritten text.

This presentation will impact the forensic science community by highlighting the problems faced by conventional methods of ink analysis (such as solubility and thin-layer chromatography) in analyzing blue gel inks and by demonstrating that brand identification of blue gel inks can be accomplished using a combination of analytical techniques.

The ability to identify a specific brand and/or batch of writing ink based on its physical and chemical properties has allowed questioned document examiners to gather a wealth of forensically useful information. For example, it may be possible to determine the earliest date of creation of a document (which may conflict with the alleged date of creation). It may also be possible to detect additions to or alterations of document by demonstrating the presence of differing ink characteristics. For many years, forensic document examiners have relied on simple tests such as the solubility of inks in different solvents, appearance of inked writings under different illuminants and the separation of dyes in thin-layer chromatography. These low-technology tests sufficed for fountain pen inks, ball pen inks and fiber-tip pen inks. However, the introduction of gel inks has required forensic document examiners to add more sophisticated (and expensive) analytical tools to their repertoires. Gel ink pens, which were patented in 1984, are environmentally friendly: organic solvents are not used in the ink formulations. Because the colorants in these pens are comprised either wholly or partly of pigments, gel pens produce writing of archival quality. The presence of pigments in these inks makes thin-layer chromatography less useful as a tool for differentiating brands of gel inks. Gel pen inks are also available in a wide range of colors and produce smoother writing than other pen inks. The qualities of gel ink make gel ink pens very marketable items, whose popularity can only be expected to grow.

In this research, thin-layer chromatography (TLC), scanning electron microscopy (SEM), visible-near infrared (VIS-NIR) reflectance spectrophotometry, and gas chromatography-mass spectrometry (GCMS) were used to analyze sixty-four blue gel ink samples. The sixty-four blue gel ink samples represented ten brands of blue gel ink pens. The blue gel pens were purchased in packs of four or more pens (as the pens are normally sold in office supply stores) and each pen in each pack was used to create a 'scribble sheet' on filter paper. The "scribble sheets" were used in all of the analyses. The VIS-NIR reflectance and GCMS data were subjected to k-means cluster analysis, principal component analysis (PCA), and hierarchical cluster analysis. The factors extracted by PCA were used to construct discriminant functions that could be used to assign writing made with blue gel pens to particular brands. The VIS-NIR reflectance measurements produced five distinct clusters of gel pens. Of the five, three were comprised of only one ink, one cluster contained two ink brands and the largest cluster

contained five ink brands. When the gas chromatograms and mass spectra (GC-MS) of the samples were compared visually, the ten different brands of blue gel pens could be broken down into seven groups, depending on the presence or absence of particular peaks in the gas chromatograms. When GC-MS data were analyzed by PCA three clusters of inks were identified. However, only one cluster was comprised of a single ink brand. The additional clusters analyzed were comprised of five and four samples, respectively. The discrepancy between visual assessment of the gas chromatograms and PCA illustrates a limitation of PCA as tool for data reduction. Further analysis of the data determined that GC-MS and VIS-NIR reflectance measurements could be successfully combined to differentiate among brands of blue gel ink. Furthermore, the discriminant functions produced from the VIS-NIR reflectance data and the GCMS data can be used to assign an unknown ink sample to its correct brand. When VIS-NIR reflectance spectrophotometry, GC-MS, TLC, and SEM were used together, all ten blue gel ink brands could be successfully differentiated. Future research will focus on adding to the brands of blue gel pens and on assessing the effect of storage conditions on the gel ink samples.

**Gel Pens, Spectrophotometry, GCMS**

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**J16**      **The Characterization of Vehicles Found in Ballpoint Ink Cartridges of Different Composition and Possible Implications on Ink Aging Examinations**

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The goal of this presentation is to provide a greater understanding of the aging characteristics of ballpoint inks, as well as, to disseminate additional information regarding the possibility of classifying the cartridge type within a writing instrument based on the presence or absence of certain chemical compounds.

This presentation will impact the forensic science community by leading to a greater understanding of inks for document examiners and researchers.

All writing inks, in their basic form, are mainly composed of a colorant(s) that is suspended in a vehicle (solvents and resins). The vehicle is the fluid portion of an ink that suspends and delivers the colorant to the substrate. Once on the paper, the solvent in ink evaporates over a period of time causing the colorant to dry onto the paper. Glycols, alcohols, and water are the most commonly found solvents in use for pens today. The choice of which solvent to use often relies on properties related to the writing instrument. The writing instrument type (e.g., fountain pen, ballpoint, felt tip marker), the composition of the ink cartridge, the region of sale (e.g., dry, humid), and other considerations all factor in to the decision of which solvents are utilized.

The composition of an ink cartridge is a consideration by manufacturers when selecting solvents for ink formulations and can be plastic-based (e.g., polyvinylchloride, polyethylene, polypropylene) or metal (e.g. brass, stainless steel). As an example, benzyl alcohol cannot be used solely in polyethylene cartridges since it diffuses through the material causing the ink to dry. The detection of certain components in questioned ink entries may suggest the type of pen cartridge used to create the writing. This information may be helpful in reducing the potential population of writing instruments, especially in cases that involve the comparison of a suspect pen with a questioned document.

The analysis of solvents for the purpose of dating inks is well documented and has been reported in the literature for over two decades. More recently, studies have indicated that certain inks can be

characterized as fast or slow aging. It is likely that aging parameters are directly affected by the combination of vehicle components. Therefore, it was hypothesized that the combination of ingredients found in the vehicle portion of an ink may be used to predict whether an ink is slow or fast aging.

In this study, research focused on characterizing the various solvents used in "plastic" and "metal" cartridges that are commonly used in ballpoint writing instruments. An analysis using gas chromatography/mass spectrometry, coupled with thermal desorption, was carried out on 100 cartridges (50 plastic and 50 metal) to determine if certain solvents, or a combination, are characteristic of cartridges having different compositions. In addition, an analysis was conducted on the inks after six months of drying to ascertain the significance of vehicle composition on the aging of ink.

The results from this study will lead to a greater understanding of inks based on their composition of the vehicle portion of ballpoint writing inks. The potential uses for this information may have a significant impact on the conclusions rendered in forensic reports. In addition, determining the age of inks based on solvent evaporation in written entries has been on the forefront in recent publications. Additional knowledge regarding the effects of vehicle components on the aging parameters of an ink will be invaluable to the forensic community.

**Writing Inks, Ink Aging, GC/MS**

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**J17**      **The Classification of Inkjet Inks Using AccuTOFä DARTä (Direct Analysis in Real Time) Mass Spectrometry**

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The goal of this presentation is to propose a new methodology for the identification of inkjet inks.

This presentation will impact the forensic science community by presenting a more recent instrumental analysis technique that can be used for the analysis of inkjet inks.

Identifying the make and model of an inkjet printer based on the examination of a questioned document can be invaluable information. However, there are numerous models of printers that can utilize the same ink, but this should not deter the forensic document examiner from narrowing the scope of possible printers. In addition, further physical examinations may help narrow the population of candidates even more. With the advent of inkjet technology, there has been a technological evolution of inkjet printers with respect to quality and speed, resulting in necessary changes to the chemistry of the ink. Indeed, this has created a time line of introductory dates for new formulations. Of course, this information can be used to ultimately determine if a document was produced on its purported dates by comparing with known standards.

AccuTOF™ DART™ is a mass spectrometer that allows spectra to be obtained by placing a sample directly in the path of the ion source. The methodology can be virtually non-destructive and involves very little sample preparation. Its use has been well documented in the recent literature with promising results for the analyses of various materials. More specifically, analyses on ballpoint inks using DART™ has been published, and research is indicating that this technique has excellent potential for the identification of certain inks.

The objective of this study is to determine if the DART™ can be used to reliably differentiate and identify inkjet inks. The images and/or text produced from an office machine system that utilizes inkjet technology can be produced using multiple colors (e.g., cyan, magenta,

yellow, black, light cyan, light magenta). This can pose a problem for non-destructive procedures that attempt to characterize inkjet inks without chemically separating and comparing the components. The methodology designed in this study relies on the creation of single spectra profiles of individual colors, subsequently combining the spectra, and then adding the newly combined data into a searchable library.

The spectral data for various inkjet inks from major manufacturers were obtained, evaluated, and entered into an electronic and searchable database. Afterwards, an analysis of printed documents from known office machines were conducted using the DART™ and classified by manufacturer and inkjet cartridge number. Finally, a blind study was conducted to determine the validity and accuracy of the methodology for discriminating and identifying inkjet inks found on questioned documents.

**DART, Inkjet, Ink**

## J18 The Use of Filtered Lighting and Infrared Luminescence for the Evaluation of Writing Inks Analyzed Using Thin Layer Chromatography

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The goal of this presentation is to provide additional knowledge regarding the evaluation of writing inks that are analyzed using thin layer chromatography, as well as, to provide information regarding the use of a digital capture station equipped with various filters.

This presentation will impact the forensic science community by demonstrating pre-existing techniques that can be used to enhance the evaluation and demonstration aspects of thin layer chromatography examinations.

Thin layer chromatography (TLC) is one of the most widely used and generally accepted scientific methodologies employed to compare and help characterize ink formulations. Since TLC is an effective and efficient method for separating and identifying colorants and nearly all ink formulations are proprietary, forensic examinations that employ TLC analysis are invaluable. For instance, two or more questioned inks can be compared to determine if they are the same, or questioned inks can be associated to a known standard. However, it must be emphasized that TLC is only one portion of an analytical scheme, and the "profile" of an ink is only achieved using the results from a series of physical, optical, and chemical examinations.

As part of the examination process, it is sufficient to compare and evaluate the components on a TLC plate using standard light and an ultraviolet source (254 and 366 nanometers). Some writing ink formulations, produced by different manufacturers, are sometimes indistinguishable after conducting optical examinations and TLC examinations. In addition, there may be batch variations in writing inks produced by the same manufacturer that are not detectable at this level of examination. Further analysis using additional analytical techniques such as gas chromatography/mass spectrometry (GC/MS) and Fourier transform-infrared spectroscopy (FT-IR) may be warranted, but are sometimes not helpful for further discrimination. Inks that are not distinguishable or their differences may not be detectable using the prescribed analytical techniques can be classified as being in the same "family" or having the same root formulation. Indeed, one must consider

that indistinguishable inks found in different brands of writing instruments may have originated from the same source (e.g., ink wholesaler) and are chemically the same.

A variety of ballpoint and non-ballpoint inks from the same families and ink formulations from different batches that were indistinguishable following TLC analysis were further evaluated using filtered light and infrared luminescence (IRL). The images were evaluated using a Video Spectral Comparator (VSC), a digital camera equipped with special filters, and an alternate light source. Differences were detected within some of the families and between batches. Analysis using GC/MS was utilized in an attempt to identify the chemical compounds responsible for the differences. Furthermore, the filtered light and IRL examinations of inks that could not be further differentiated on TLC plates proved to be an excellent corroborating step during the evaluation process. This was especially evident in cases where resolution of bands was minimal since the various filters provided detailed contrast. The digital images of chromatographic profiles were captured and determined to be beneficial to demonstrate similarities and differences to jurors if necessary.

**Writing Inks, Thin Layer Chromatography, Filtered Lighting**

## J19 Comparison of a Standard Set of Black Ballpoint Inks Using a Direct Analysis in Real Time Mass Spectrometer (DART™-MS)

*Joseph C. Stephens, MSFS\*, Gerald M. LaPorte, MSFS, and Danna Bicknell, MSFS, United States Secret Service, Forensic Services Division, 950 H Street, NW, Washington, DC 20223*

After attending this presentation, attendees will understand DART™ and its application to forensic ink examination on questioned documents.

This presentation will impact the forensic science community by providing a potential alternative to current ink analysis techniques.

Examination of writing inks has revolved around the use of chromatography for decades. Chemists frequently use chromatography to separate components of a mixture to avoid potential interferences or overlaps in the obtained spectra. Often, this process requires some sample preparation or pre-treatment prior to analysis and results in destruction to small parts of the document. However, overlaps and interferences can be minimized, or even eliminated, if an instrument is used that is sensitive enough to distinguish between two components that are unresolved by other analytical techniques.

The Direct Analysis in Real Time Mass Spectrometer (DART™-MS) utilizes an ion source to generate a beam of electrically excited helium that is used in the analysis of complex mixtures. Instead of separating the components using some chromatographic medium, DART uses an AccuTOF™ mass spectrometer to separate the constituent ions. The time of flight spectrometer provides exact masses at accuracies up to a few millimass units. Such sensitivity, coupled with a reference collection of precisely known molecular masses, such as the National Institute of Standards and Technology (NIST) mass spectral library, allows for identification of numerous dyes, pigments, and vehicles (solvents) simultaneously without the use of chromatography. Sample preparation for DART™ is minimal and signal collection takes only a few seconds. This increased sensitivity and potential for nondestructive sample analysis make DART™ a viable option to consider as an alternative for the examination of writing inks. Previous research in academia has also been published to validate the application of DART™-MS to ink analysis.

Previous studies have been conducted in which a set of forty-four (44) black ballpoint pen inks were analyzed using a variety of techniques, including Video Spectral Analysis (VSA), Gas Chromatograph / Mass Spectrometry (GC/MS), Thin Layer

Chromatography (TLC), scanning densitometry, and Hyperspectral Imaging. This black ballpoint set was recently analyzed using the DART™-MS and the results compared with those generated using these other analytical techniques. The discriminating power of the inks was calculated based on inter-sample comparison. This research could impact the questioned document field by providing a potential alternative to current ink analysis techniques.

**Questioned Documents, Ballpoint Pen Ink, DART™**

## J20 Spectral Analysis of Canadian Currency as a Potential Tool for Counterfeit Detection

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After attending this presentation, attendees will gain an understanding of a new technique of visible - near infrared spectrometry, and its application to the detection of counterfeit Canadian currency. This presentation will demonstrate how despite the efforts of counterfeiters, this tool can differentiate between genuine and counterfeit bills at 95% and 99% confidence levels, in a non - destructive, repeatable manner.

This presentation will impact the forensic science community by illustrating a novel application of remote sensing technology that increases the confidence in detection of counterfeit Canadian banknotes in a non-destructive repeatable manner.

Counterfeits account for a substantial value of banknotes in circulation. With the introduction of sophisticated scanners, color photocopiers, and ink-jet printers in the early 1990s there was a dramatic change in counterfeiting technology. There are a number of counterfeit detection methods utilized by law enforcement agencies; however, these suffer from various drawbacks: some are destructive in their nature, some too time consuming, and some simply imprecise. In addition, there have been very few studies that focused on Canadian banknotes. For this study a novel approach to counterfeit detection was chosen, focusing on how the banknotes – both counterfeit and legitimate – reflect specific wavelengths of visible and near-infrared light. Banknotes are composed of two primary parts – the paper and the ink, both of these components affect the spectral response for each of the banknote denominations. In the case of counterfeit the paper is of a varied nature. The first part of this research compared the four most common paper types used by counterfeiters in Canada in order to observe if it is possible to differentiate between the paper types and as well as seeing how the paper type impacts the spectral signature. This analysis showed that the overall spectral shape is significantly different between all paper types and it is possible to differentiate between the paper types commonly used by counterfeiters.

In the focal part of this study the spectral signatures were compared for the $5, $10, and $20 Journey series notes and the $20 Birds of Canada series. Banknotes from each denomination, both from legitimate and counterfeit, were sampled at specific points on the bills using a handheld spectrometer operating at the 325 - 1075nm wavelength range. For all denominations the spectral signatures were found to be significantly different between the counterfeits and the real notes at both 95% and 99% confidence levels, and therefore this technique may be a rapid and reliable method to use in counterfeit detection.

**Hyperspectral Analysis, Counterfeit, Spectrometry**

## K1 The Analysis of PM Oral Swabs by SPE and LC–MSMS for Fentanyl as an Indicator of Administration

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After attending this presentation, attendees will learn about the usefulness and versatility of the oral swabs as a means of extracting fentanyl/norfentanyl from postmortem oral cavities. Attendees will also learn about the efficiency of solid phase extraction LC-MSMS methods in confirming these drugs in this matrix.

This presentation will impact the forensic science community by showing how fentanyl/ norfentanyl can be extracted/isolated and analyzed from swabs taken from the oral cavities in postmortem cases. This methodology will assist forensic toxicologists and pathologists when samples of blood and urine are limited.

The goal of presentation is to show how useful oral swabs taken at postmortem examination can be for the analysis of fentanyl/norfentanyl in cases when limited samples are available to analysts, forensic toxicologists, and forensic pathologists. The levels of the fentanyl (and nor fentanyl) found on the swabs are referenced against the values obtained by toxicological analysis of postmortem blood for the same cases. The data presented should add another method of analysis for facilities providing toxicological services.

In 2007-2008, oral swabs were taken from 72 post mortem cases by the pathology staff at Erie Co. Medical Examiner's Office New York where fentanyl was related to the case. In each of the cases, two swabs were employed simultaneously to extract samples from the oral cavities. The swabs were forensically sealed and submiited to Northern Tier Research (NTR). The swabs from each case were split and half of the samples were sent to Massachusettss State Police Crime Laboratory (MSPCL). These samples were used to confirm Northern Tier Research findings. Following submission to the respective laboratories, the oral swabs were extracted with 200 µL of methanol for 30 minutes in a sample tube containing fentanyl-d5/norfentanyl-d5, the swabs were washed further with a 100 µL of methanol. The swabs were removed from the sample tube before 2 mL of phosphate buffer (pH 6) were added. This solution was extracted by solid phase extraction using a mixed mode ($C_8$/SCX) column (200 mg, 6 mL). The SPE columns were conditioned with methanol, DI water, and ph 6 phosphate buffer (3 mL, 3 mL, 1 mL, respectively). After washing with DI water, 0.1 M acetic acid, and methanol (3 mL of each), the SPE columns were dried and eluted with: (NTR): 3 mL of ethyl acetate/acetonitrile/ammonia (78:20:2) and (MSPCL): 3 mL of dichloromethane/ isopropanol/ ammonia (78:20:2). The eluates were evaporated to dryness and reconstituted in methanol for analysis by LC-MSMS using 5µL for injection.

At NTR, tandem mass spectrometry was performed in MRM (Fentanyl: 337.2-> 188.1/105.7, Norfentanyl: 233.0->84.0/55.1) with a 50 x 2.1mm (3 µm) phenyl column. At MSPCL tandem mass spectrometry was employed in the same MRM mode using a 50 x 2.1mm (5 µm) $C_{18}$ column. Chromatography was performed with a gradient program of acetonitrile and 0.1% aqueous formic acid at each laboratory.

Calibrators and controls were set up by extracting 0, 1, 2, 5, 10, and 7 ng of fentanyl/norfentanyl from aqueous buffer samples (2 mL) by the individual procedures. From the analysis of the calibrators and controls: $r^2$ value> 0.995, recoveries > 90% (NTR/ MSPCL), and a limit of detection of 0.1 ng/ mL, respectively were acheived.

Of the 72 postmortem cases where oral swabs were taken, six were confirmed to be positive for fentanyl. In two of the six cases, both fentanyl and norfentanyl levels greater than 1 ng were confirmed by LC-MSMS in both forensic laboratories (NTR/ MSPCL). These six cases were shown to have fentanyl and norfentanyl levels in blood ranging from 0.8 ng/ mL to 10.5 ng/ mL for fentanyl and 0.8 ng/ mL to 30.8 ng/ mL for norfentanyl, respectively. This data was obtained by the forensic toxicology laboratory, Erie Co. NY.

Based on data presented, analysts, forensic toxicologists and pathologists involved in post mortem cases where fentanyl and norfentanyl is suspected may wish to consider the usefulness of oral swabs in their analytical protocols. Although no direct correlation between the concentration of the drugs found in blood and those obtained from oral swabs can be drawn, this study has shown that in those cases where fentanyl was postive in oral swabs, it was confirmed in the corresponding blood samples. This relationship may be very useful in postmortem fentanyl cases.

**Fentanyl, SPE, LCMSMS**

## K2 Rapid Quantification of THC and Carboxy–THC in Blood by LC–MS/MS

*Albert A. Elian, MS\*, Massachusetts State Police Crime Lab, 59 Horse Pond Road, Sudbury, MA 01776*

After attending this presentation, attendees will understand a simple and improved solid phase extraction (SPE) method for analyzing THC and THC-COOH in whole blood.

This presentation will impact the forensic community by assisting forensic toxicologists/analysts in implementing a simple solid phase extraction procedure coupled with LC-MS/MS for low level quantification of THC and THC-COOH in whole blood samples.

In this procedure, after the addition of the internal standards (D3-THC and D3-THC-COOH) to 1 ml of whole blood, 2 mL of ice cold acetonitrile were added dropwise while mixing. The samples were allowed to stand for 10 minutes, after which the samples were centrifuged (10 minutes at 3000 rpm). Each supernatant was decanted into a clean tube and mixed with 5 mL of pH 7 phosphate buffer (0.1 M) prior to solid phase extraction. The mixed mode SPE columns ($C_8$/ SAX) were conditioned with methanol, pH 7 buffer (3, 3, mL, respectively) after which, the samples were loaded. The SPE columns were washed with 3 mL DI water, dried, and washed again with 3 mL hexanes then dried again for 5 minutes under full vacuum. Following elution of THC / THC-COOH with 2 mL of hexane: ethyl acetate (1/1), the eluents were collected and evaporated to dryness. The residue was reconstituted with 100 µl of the mobile phase solution.

Liquid chromatography was performed using $C_{18}$ column (50x 2.1mm, 5 µm), at 0.55mL/min flow using a gradient program. The mobile phase program: (A) 0.1% aqueous formic acid) / (B) acetonitrile containing 0.1% formic acid was started at 50% (B) for 0.5 min, increasing to 90% (B) over 1.5 minute, and holding at 90% B for one minute before returning to 50% (B) and equilibrated for 2 minutes. The total chromatographic run time for each analysis was 4.5 minutes

including equilibration time. MS/MS analysis was conducted using a tandem mass spectrometer equipped with ESI in negative ion mode for THC-COOH/ D3-THC-COOH and was operated with multiple reaction monitoring (MRM) under the following conditions: curtain gas 15, collision gas medium, ion spray voltage -4500V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 343.1 → 299.3 and 245.3 for THC-COOH, and m/z 346.1 → 302.3 and 248.3 for D3-THC-COOH. Positive ion mode was employed for THC/ D3-THC under the following conditions: curtain gas 15, collision gas medium, ion spray voltage 5000V, temperature 650 °C, ion source gas(1) 50, ion source gas (2) 50. The following transitions were monitored (quantification ions underlined): m/z 315.2 → 193.2 and 123.1 for THC, and m/z 318.2 → 196.2 and 123.1 for D3-THC.

Linearity (r² >0.99) was achieved from 0.25 ng/mL to 50 ng/mL, (THC/ THC-COOH) and the limits of detection were determined to be 0.1 ng/mL for THC and 0.25 ng/mL for THC-COOH, respectively. The limits of quantification were 0.25 ng/mL for THC and 0.5 ng/mL for THC-COOH, respectively. Recoveries were > 92% for THC and > 87% for THC-COOH, respectively measured at a target value of 4.0 ng/mL. Intra and inter-day precision was less than 7% and 11%, respectively for THC and less than 8% and 12%, respectively for THC-COOH. Ion suppression studies revealed that suppression of monitored ions was less than 6%.

This SPE method coupled with and fast LC-MS/MS provides a simple, sensitive, and reproducible quantitative method for the analysis of THC and its primary metabolite in whole blood. This procedure should be of great assistance to those analysts actively involved with the LC-MS/MS analysis of these drugs in biological matrices.

**THC and Metabolite, Solid Phase Extraction, LC-MS/MS**

# K3 Simultaneous Quantification of Twenty Common Drugs of Abuse and Metabolites in Human Meconium by LCMSMS

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After attending this presentation attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

This presentation will impact the forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings.

Drug abuse during pregnancy is associated with adverse obstetrical and neonatal outcomes. Detection of *in utero* drug exposure is often accomplished by meconium analysis due to ease and non-invasiveness of specimen collection and a long window of drug detection. However, the amount of meconium is often limited, prohibiting multiple assays for different drugs of abuse. Attendees will be introduced to a liquid chromatography tandem mass spectrometry (LCMSMS) method for simultaneous quantification of common drugs of abuse in human meconium.

An LCMSMS method for the simultaneous quantification of amphetamine (AMP), methamphetamine (MAMP), *p*-hydroxymethamphetamine (pOHMAMP), cocaine (COC), benzoylecgonine (BE), cocaethylene (CE), *m*-hydroxybenzoylecgonine (mOHBE), nicotine (NIC), cotinine (COT), 3'-*trans*-hydroxycotinine (OHCOT), morphine (MOR), 6-acetylmorphine (6AM), codeine (COD), hydromorphone (HYM), hydrocodone (HYC), oxycodone (OXY), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine (BUP), and norbuprenorphine (NBUP) in meconium in only 0.25 g of meconium was developed and validated.

Meconium specimens (0.25 g) fortified with deuterated internal standards were homogenized in acidic methanol. After centrifugation and supernatant evaporation, analytes were isolated using mixed mode solid phase extraction and analyzed by LCMSMS operating in positive multiple reaction monitoring (MRM) mode. Two analytical runs utilizing the same extract were required: a 5-µL injection, 18 minute run with gradient elution that quantified all analytes except BUP and NBUP. These two analytes were measured in a second 5 min isocratic run with a 10-µL injection volume to enhance sensitivity. The analytical method was validated over four days for limits of quantification, recovery, imprecision, extraction efficiency, matrix effects, carryover, and endogenous and exogenous interference.

Limits of quantification were 1 ng/g for COT, CE, BE, and COC, 2.5 ng/g for MAMP, EDDP, MTD, and pOHMAMP, 5 ng/g for AMP, mOHBE, NIC, OHCOT, MOR, 6-AM, HYM, HYC, OXY, and 25 ng/g for BUP and NBUP. The upper limit of quantification for all analytes was 500 ng/g, except for pOHMAMP at 250 ng/g. Correlation coefficients for each calibration curve were >0.996 with all calibrators quantifying within ±20% of target when calculated against the calibration curve. Validation parameters were tested at three concentrations spanning the linear dynamic range. Intra- and inter-day recovery ranged from 83.3 – 126.6% and 80.1 – 129.0%, respectively. Inaccuracies of up to 30% were considered acceptable due to meconium's complexity. Intra- and inter-day imprecision ranged from 0.9 – 16.9% relative standard deviation (RSD) and 3.1 – 9.8% RSD, respectively. Extraction efficiencies ranged from 46.7 – 96.0%. Matrix effects ranged from -305.7 – 40.7%, depending on the analyte, with negative values indicating ion enhancement. Matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds, highlighting the importance of employing matched deuterated internal standards in LCMSMS quantification procedures, especially with complex matrices. Similar results were observed for matrix effects determination in seven different blank meconium sources fortified with low quality control concentrations; while matrix effects varied between meconium specimens, matrix enhancement or suppression of related native, and deuterated compounds were similar and quantification was within acceptable limits. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 48 h on the 15°C autosampler. Losses of less than 34.0% were observed for each condition, except for 6AM and MOR. After room temperature, 4°C, and three freeze-thaw cycles, up to 85.8% of 6AM was lost; however, MOR concentrations under these conditions increased by up to 31.2%. In cases of suspected heroin exposure, meconium should be immediately frozen and repeated freeze thaw cycling should be avoided. No analyte carryover was observed at two times the upper limit of quantification. No interference by 57 illicit and therapeutic drugs or endogenous meconium compounds was observed. Method applicability for all analytes except 6AM, BUP, and NBUP was demonstrated by analysis of meconium from drug-exposed neonates.

The most comprehensive chromatographic method for the identification and quantification of drugs and metabolites in meconium is described. This LCMSMS method will impact the clinical and forensic community by offering a novel analytical method for sensitive and specific simultaneous quantification of 20 analytes in a single extraction and small meconium specimen, offering time and resource savings. This method will be employed in prenatal drug exposure

studies to correlate meconium concentrations to neonatal outcome measures.

**LCMSMS, Drugs of Abuse, Meconium**

## K4 Analysis of Ethylglucuronide (EtG) and Ethylsulfate (EtS) in Urine by Liquid Chromatography Mass Spectrometry

*Sandra Valtier, PhD\*, Clinical Research Division, 2200 Bergquist Drive, Building 4430, Lackland AFB, TX 78236*

The goal of this presentation is to present a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) method for quantitative analysis of the alcohol biomarkers, ethylglucuronide (EtG), and ethylsulfate (EtS) in urine.

This presentation will impact the forensic community by providing data obtained from a method validation study of urinary EtG, and EtS by LC/MS/MS. This study evaluated sensitivity, linearity, precision, interference, and other related parameters associated with method validation.

Measurement of ethanol in breath, blood, or urine is used in detecting recent alcohol consumption; however, ethanol is rapidly cleared from the body making it difficult to use as an indicator of alcohol use disorder. On the other hand, alcohol biomarkers, EtG and EtS, can be detected for a longer period of time making them more suitable indicators of alcohol consumption or exposure and potentially as indicators of alcohol use disorder. Samples were analyzed on a liquid chromatography system coupled to Applied Biosystems triple quadrupole mass spectrometer.

Standards spiked with concentrations of EtG and EtS ranging from 10 - 10,000 ng/mL were prepared in mobile phase and in alcohol negative urine. Urine samples (n = 14) collected from subjects following alcohol consumption were also evaluated. The LC column used for this evaluation was the Thermo Electron Corporation Hypercarb. In a previous study, the Hypercarb column showed the best chromatography for analysis of EtG and therefore was used for analysis of both markers. The LC mobile phase consisted of 5% acetonitrile with 0.1% formic acid; flow rate was set at 0.5 mL/minute. The working internal standard solution contained 550 ng/mL EtG-D5/100 ng/mL EtS-D5 in mobile phase. A 10 µL aliquot of standard or urine was mixed with 90 µL of internal standard solution. The samples were analyzed on Applied Biosystems 4000 QTrap LC/MS/MS system. The mass spectrometer was set in the ESI negative mode and analysis was performed using multiple reaction monitoring (MRM). The MS/MS ion transitions monitored were $m/z$ 221 → 75 and 221 → 85 for EtG; $m/z$ 124.9 → 79.9 and 124.9 → 96.9 for EtS; $m/z$ 226 →75 for EtG-D5 and $m/z$ 130 → 98 for EtS-D5.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 10 to 10,000 ng/mL EtG and EtS prepared in mobile phase and in urine. Values were considered within acceptable range if the measured amount was within ± 20% of target concentration and ±20% of ion ratio calculation. The linear range was shown to be 10 to 10,000 ng/mL for EtG and 10 to 5,000 ng/mL for EtS with a LOD and LOQ of 10 ng/mL for both analytes. The method yielded good precision for both urine and mobile phase prepared samples with RSDs of < 5% for both EtG and EtS. The present study provides a simple and rapid validated LC/MS/MS method for quantitation of the alcohol biomarkers, EtG and EtS, in urine.

**Ethylglucuronide, Ethylsulfate, LC/MS/MS**

## K5 Validation of Opiate Detection and Quantification in Human Urine Using Liquid Chromatography and Tandem Mass Spectrometry (LC/MS/MS)

*Chelsy L. Wingate, BS\*, 1517 Hawk Tree Drive, College Station, TX 77845*

After attending this presentation, attendees will have assessed the validity of a new method used in the detection and quantification of opiates in urine specimens using LC/MS/MS.

This presentation will impact the forensic community by demonstrating the development of a highly sensitive method for detection and quantification of opiates that provides rapid results, which can be utilized in both clinical and forensic toxicological settings.

The abuse of prescription pain medication has increased dramatically over recent years. Opiates, which have a high potential for addiction, are among several classes of drugs commonly used in the treatment of chronic pain. With the growing amount of opiates being used to treat pain, it is important for physicians to have the ability to monitor patient prescription use to determine if abuse has occurred. A new highly sensitive method has been developed that detects the presence of opiates in human urine specimens using liquid chromatography and tandem mass spectrometry (LC/MS/MS). This method can analyze a large quantity of samples in a short period of time due to simple sample preparation and online extraction. Alternative methods such as Gas Chromatography/Mass Spectrometry (GC/MS) require longer sample preparation time given that the sample must be extracted from the biological matrix before analysis can occur. The liquid chromatography instruments used to perform this study are multiplex systems having two to four injection ports (Thermo Scientific Aria TLX2 and TLX4) coupled with a triple quadruple mass spectrometer (TSQ Quantum Access). This multiplex system allows for analysis of a much larger number of samples than standard LC/MS/MS systems and the combination of the LC system with tandem mass spectrometry eliminates the need for derivatization, also decreasing analysis time.

In order to report toxicological results, it is crucial that the method utilized can provide reliable, reproducible results. A validation study was performed to assess the ability of this method to detect and quantify opiates accurately in urine specimens. The opiates analyzed include morphine, oxymorphone, hydromorphone, codeine, oxycodone, and hydrocodone. These six analytes are the most common opiates used in prescription pain medication. T he validation parameters evaluated in this study consist of accuracy, inter and intra-assay precision, linearity, carryover, lower and upper limit of quantification, limit of detection, and specificity.

The linearity or calibration model contained ten calibrators ranging from 50ng/ml to 50,000 ng/ml and all analytes produced an $R^2$ value above 0.99. The precision and accuracy was performed by analyzing five replicates at three concentrations. The precision study was performed over a three day period on two different instruments. The % CV was calculated for each day and was not to exceed 10%. All of the analytes passed this criterion except for morphine on days one and two with the lowest concentration having a CV of 13.7% and 11.94% as well as hydrocodone on day one with a CV of 11.08% for the highest concentration. Accuracy calculated based on the value determined by analysis and the true value of each analyte. All of the analytes were within 10% of the target except for oxymorphone on day one for all three concentrations and oxycodone on day two for the middle concentration with a percent accuracy of 112%. Oxymorphone is the least stable of the six opiates.

The lower limit of quantification for all six analytes was determined to be 100 ng/ml where as the limit of detection was determined to be 50

ng/ml. The upper limit of quantitation was found to be 50,000 ng/ml for all of the analytes and minute carryover was observed from extremely high concentrations of the analytes. Several different drugs such as acetaminophen, 6-acetylmorphine, methadone, buprenorphine, norbuprenorphine, morphine-3-glucuronide, oxazepam, lorazepam, alprazolam, α-hydroxyalprazolam, diazepam, and nordiazepam were analyzed for interference purposes It was determined that 6-acetylmorphine gave positive results for morphine. These results are most likely due to the acid hydrolysis step required for deconjugation. To avoid conversion from 6-acetylmorphine to morphine, it may be advantageous to use an enzyme such as beta glucuronidase for hydrolysis rather than an acid. The results from this study indicate that this LC/MS/MS method can provide reliable, reproducible results for the detection and quantification of opiates in a short period of time.

**Liquid Chromatography Coupled With Tandem Mass SPE, Method Validation, Opiates**

## K6 Quantitative Determination of SSRI Drugs and Metabolites in Human Plasma by SPE-LC-MS/MS

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After attending this presentation, attendees will obtain valuable information about an improved, accurate, sensititve, and specific method for the quantitative analysis of the non-tricyclic class of anti-depressant drugs in human biological samples obtained from suicide decedents.

The presentation will impact the forensic community by significantly advancing our knowledge regarding the prior use of SSRI drugs in suicide decedents. This information will be a critical element in building a community-based treatment approach to preventing suicides.

Selective serotonin re-uptake inhibitors (SSRIs), a class of non-tricyclic antidepressants, are marketed as safe and effective in treating depression, anxiety disorders, and some personality disorders. Although, questions related to their safety were raised, with studies reporting a possible association with suicidal tendencies, inferences regarding the validity and strength of such an association have been divergent. The goal of this study was to develop a rapid and sensitive HPLC-MS/MS/ESI method for simultaneous determination and screening of the most commonly prescribed SSRIs in human plasma samples from suicide decedents.

A solid phase extraction (SPE) method coupled to LC-MS/MS was developed for the simultaneous analysis of 5 SSRIs, Fluoxetine (Fluox), Paroxetine (Parox), Fluvoxamine (Fluvox), Sertraline (Sert), and Citalopram (Citalo), and three of their pharmacologically active N-demethylated metabolites, Norfluoxetine (Norflux), Norsertraline (Norsert), and N-desmethylcitalopram (Descitalo), using Waters Oasis HLB SPE cartridges. Stock solutions of the individual drugs, as well as the internal standards (I.S.), Fluox-$d_6$, Norfluox-$d_6$, Parox-$d_6$, Sert-$d_3$, Norsert-$d_4$, and Citalo-$d_6$, for calibration standards and QC were prepared in MeOH and stored at -20°C.

An LC system consisting of Agilent HP 1100 series and a Thermo/Finnigan Quest TSQ triple-stage quadrupole MS, equipped with Xcalibur (v 1.1) operating software was used for data analysis. Ionization was achieved using electrospray in the positive ionization mode. Chromatographic separation of all the compounds was achieved within 15 mins using a Waters YMC ODS-AQ C18 (150×2 mm, 3 μm) analytical column, and a mobile phase gradient consisting of 0.1% formic acid in water and MeOH at 10%, 30%, 40%, and 10% for 1, 1, 4,

and 9 min, respectively. Identification and quantification were based on selected reaction monitoring.

Fluox, Parox, Fluvox, Sert, Citalo, Norfluox, Norsert, Descitalo, Fluox-$d_6$, Norfluox-$d_6$, Parox-$d_6$, Sert-$d_3$, Norsert-$d_4$, and Citalo-$d_6$ were detected by measuring transitions of m/z 310→ 148, m/z 330→ 192, m/z 319→ 200, m/z 306→ 159, m/z 325→ 262, m/z 296→ 134, m/z 292→ 159, m/z 311→ 262, m/z 316→ 154, m/z 302→ 140, m/z 336→ 198, m/z 309→ 159, m/z 296→ 160, and m/z 331→ 262, respectively. To evaluate linearity, three calibration curves over a concentration range of 1–1000 ng/mL for each of the compounds, were tested separately. A $1/X^2$ weighted quadratic curve was used for quantification. The method was fully validated, including inter- and intra-run accuracy (within 15% of target concentration) and precision (CVs <15%) for QC samples at 5, 50 and 300 ng/mL. The mean recovery for all SSRI drugs ranged from 32-74%. Stability testing showed no evidence of degradation in processed plasma samples during 3 successive freeze/thaw cycles or after storage at -20°C for at least four weeks or at 4°C after at least 24-48 hrs.

The method described herein is accurate, sensitive, highly specific, and can be used for routine therapeutic drug monitoring, toxicological screening, as well as for the study of the pharmacokinetics and metabolism of the SSRI drugs in biological specimens from normal subjects, as well as from suicide decedents.

**Anti-Depressants, SSRI Drugs, LC-MS/MS**

## K7 Evaluation of Inter-Instrument Transferability of LC/MS/MS Methods

*Tania A. Sasaki, PhD\*, Applied Biosystems, 850 Lincoln Centre Drive, MS 430, Foster City, CA 94404; and Adrian Taylor, MS, and Adam Latawiec, MS, MDS Analytical Technologies, 71 Four Valley Road, Concord, Ontario L4K 4V8, CANADA*

After attending this presentation, attendees will have learned the advantages and limitations of direct transfer of LC/MS/MS methods across instruments and across labs and the considerations for successful transfer of methods.

This presentation will impact the forensic community by investigating data quality when using a method from one lab and directly transferring the method to another lab. Direct transfer of methods decreases the amount of time required to implement an analytical assay in a laboratory.

The objective of this paper is to develop an LC/MS/MS method in one lab and directly transfer that method to other labs which have the same make/model of LC/MS/MS system without further optimization. Data are analyzed and variations are compared across instruments and labs.

An LC/MS/MS method was developed to detect and quantify several different drug compounds across various drug classes. After method development, the method was directly transferred to 4 different laboratories with the same model of LC/MS/MS instrument; no additional tuning or optimization of the system was performed. The inter-instrument data was analyzed and the consistency of the data evaluated. Sensitivity, ruggedness, and reproducibility were all compared.

Data analysis showed that direct transfer of an LC/MS/MS method between different instruments was possible. When sensitivity of the method was evaluated, all systems were within about 3x of each other. The biggest variable was retention time of the analytes, as it is necessary to consider several factors, such as tubing length, mobile phase consistency, and column-to-column reproducibility.

This study showed that it is feasible to develop methods and directly transfer these methods between laboratories to other instruments of the

same model. No significant variations in sensitivity or other aspects of data quality were observed. The ability to transfer methods without individual optimization of each instrument can save substantial time in method set-up and implementation.

LC/MS/MS, Method Development, Toxicology

## K8 Determination and Quantitation of Noroxycodone in Human Urine Samples Using High Performance Liquid Chromatography - Electrospray Ionization-Tandem Mass Spectrometry

*Christopher Doctorman, BS\*, University of Central Oklahoma, 100 North University, Edmond, OK 73034; and Chelsy L. Wingate, BS, 1517 Hawk Tree Drive, College Station, TX 77845*

After attending this presentation, attendees will have a greater understanding of opiate chemistry, metabolism, kinetics, and pharmacology, as well as be familiar with and implement current LC/MS/MS technology. Attendees will also gain information about an analytical method for determination of noroxycodone, a metabolite of oxycodone, and will understand the metabolic pattern for oxycodone.

This presentation will impact the forensic community by giving greater insight into human metabolism of oxycodone. This information can be utilized to perfect or improve current methods for detecting and quantifying oxycodone and its metabolites in clinical and forensic toxicological settings.

Oxycodone (4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one), is an analgesic, semi synthetic opioid derived from thebaine. Also known by its manufactured names OxyContin™, OxyNorm™, Roxicodone™, and others, it comes in a variety of shapes and dosages. Oxycodone is commonly prescribed for significant pain management typically associated with cancer, and has been used clinically for this purpose in the United States for the past eighty years. It has been a "drug of abuse" for nearly 50 years.

Oxycodone is metabolized in the body by two isoenzymes Cytochrome P450 (CYP) 3A4 and CYP2D6. CYP3A4-mediated metabolism of the compound yields N-demethylated metabolites noroxycodone, noroxymorphone, and a and b noroxycodol. CYP2D6-mediated metabolism produces O-demethylation of oxycodone to oxymorphone and a and b noroxymorphol, and 6-keto-reduction to a and b oxycodol.

Human urine samples, collected as part of another study to determine the elimination rate of oxycodone, were used as test samples for the detection and quantitation of noroxycodone. A method developed for the simultaneous quantitation of several opiates, including codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and morphine, was modified to also incorporate noroxycodone as one of the compounds using selected ion monitoring (SIM). This method was utilized on a 4-channel multiplexing HPLC system interfaced with triple quadrupole mass spectrometer. Limit of quantitation, as well as between day accuracy and precision (%deviation and %CV) of noroxycodone was established at 100 ng/mL (3.9% and 24.9%).

Urine samples were collected over a period of a week from seven individuals given one of three different concentrations of oxycodone, along with a naltrexone blockade (50 mg per day). Concentrations of noroxycodone, oxycodone, and oxymorphone resulting from the analysis of an individual dosed with 80 mg tablets of oxycodone have shown noroxycodone to be the primary metabolite (70.8%±4.7) followed by oxycodone (18.5%±5.2) and oxymorphone (10.8%±2.1). Results for samples from other individuals will be tabulated and presented. These concentration results indicate that CYP3A4 mediation is the predominant metabolic pathway of oxycodone in humans.

Noroxycodone, High Performance Liquid Chromatography, Tandem Mass Spectrometry

## K9 Development and Validation of a LC/MS Method for the Determination of Guanfacine in Urine

*Sara J. Kester-Florin, BS\*, 944 Wye Drive, Akron, OH 44303; and Carl E. Wolf, PhD, and Alphonse Poklis, PhD, Medical College of Virginia, Box 98-165, VCU/MCVH Station, Richmond, VA 23298-0165*

After attending this presentation, attendees will become familiar with a validated liquid chromatography/mass spectrometry (LC/MS) method for detecting and quantifying guanfacine in urine specimens.

Guanfacine is a drug that was initially approved for the treatment of hypertension in adults, but has been recently approved (2007) for the treatment of attention deficit/hyperactivity disorder (ADHD) in adolescents. Due to the new therapeutic use, an increase in both availability and consumption of this drug required the development of an analytical method to detect the use or abuse of guanfacine. A validation of this LC/MS method will impact the forensic community by providing the field of toxicology with a rapid, robust analytical method that requires a small sample volume, and is also sensitive enough to detect drug use at a therapeutic dose.

The validation of a LC/MS method for the detection and quantification of guanfacine in urine is presented. Guanfacine was extracted from alkaline buffered urine using a liquid-liquid extraction scheme with ethyl acetate. Two hundred microliters of samples, controls, and calibrators were prepared with the addition of 10µL of protriptyline internal standard (2mg/L). Samples were buffered to a pH of 9.5 with 200µL saturated carbonate:bicarbonate solution. Five hundred microliters of ethyl acetate was added to the samples, followed by two minutes of rotation and five minutes of centrifugation at 3000rpm. The organic layer was transferred to a clean test tube, evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 200µL of mobile phase. Guanfacine and protriptyline were separated and quantified on a reverse phase S-5 micron, 2.0 x 150mm column in a high performance liquid chromatography (HPLC) separations module coupled to a mass spectrometer (MS) with electrospray ionization operated in the positive ionization mode. The mobile phase consisted of 40% 10mM ammonium formate in methanol, and was delivered isocratically at a flow of 0.3 mL/min. Sample injection volume was 10µL. The MS was operated in selected ion resonance mode (SIR) using the following m/z ions: 246, 248, and 250 for guanfacine, and 264 and 265 for protriptyline. Under these conditions the retention time for guanfacine and protriptyline were 2.1 min and 3.6 min, respectively.

The analytical measurement range for guanfacine ranged from 5ng/mL to 2000ng/mL with a 5ng/mL limit of detection (LOD) and a 20ng/mL limit of quantitation (LOQ). The method was shown to be both precise and accurate. Precision for the assay was determined at concentrations of 40ng/mL, 100ng/mL, and 500ng/mL, (n=6), the %CV was <15% for all three concentrations. Percent recovery of guanfacine was also performed using the same concentrations and was shown to be 92%, 89%, and 93% respectively. Interference with other therapeutic drugs and drugs of abuse was assessed by analyzing two controls containing known concentrations of drugs in both categories. No interferences were noted. The method was used to analyze over 100 random post diagnostic specimens from children ranging in age from 4-

to 18-years-old. Ten of the samples yielded positive results for the presence of guanfacine. Some of these samples were evaluated using a previously validated gas chromatography/mass spectrometry (GC/MS) method, and results were found to be comparable. Of the ten positive samples, seven were confirmed to be from patients who were prescribed guanfacine. No patient history was available for the remaining samples. This LC/MS method provides a rapid and reliable method for the routine determination and quantitation of guanfacine in urine specimens.

**Guanfacine, LC/MS, Electrospray Ionization**

## K10 The Analysis of Pain Management Drugs Found in Urine Samples by LC/MS/MS

*Greg A. Newland, BS*, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404*

After attending this presentation, attendees will learn about a new drug screening test for the analysis of pain management drugs using LC/MS/MS technology.

This presentation will impact the forensic community by providing information that enables toxicologists to easily test for a large list of drugs that are used for management of acute and chronic pain.

A multitude of drugs have historically been used to ease the pain patients suffer with conditions ranging from cancer to arthritis. As a result many labs, both clinical and forensic, have been looking for an application to test for all of the major drugs used during the treatment of these conditions. This application covers the testing of these drugs in urine matrix by "dilute and shoot" type sample prep. The use of LC/MS/MS allows the user to do limited sample prep while still providing adequate specificity to test for more than 40 different pain management drugs in less than 8.5 min from injection to injection.

All drugs were analyzed in a single injection using a LC/MS/MS and were extracted from urine after an enzyme hydrolysis. The Limits of Quantitation differed for each drug but ranged from <5 ng/ml to 200ng/ml when extracted using a 1:10 dilution of urine samples. The linearity for each drug spiked into urine exceeded R correlation of 0.98. Each drug was analyzed using two transitions and the LOQ was based on the least sensitive of the two transitions. Ion Ratios were calculated for each ion and were <40% at the LOQ of each ion.

**LC/MS/MS, Pain, Urine**

## K11 A Quick LC/MS/MS Method for the Analysis of Common Benzodiazepines and Opiates

*Tania A. Sasaki, PhD*, and Claire J. Bramwell-German, PhD, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404; and Sumandeep Rana, MS and Wayne B. Ross, MCLS, Redwood Toxicology Laboratory, 3650 Westwind Boulevard, Santa Rosa, CA 95403*

After attending this presentation, attendees will understand LC/MS/MS and its utility as an analytical technique to detect use or abuse of benzodiazepines and opiates.

This presentation will impact the forensic community by teaching about a method that is easier and has a faster turnaround time than many techniques in use today.

The objective of this paper is to develop a fast method for analysis of common opiates and benzodiazepines in urine. The method presented has a faster run time, simple sample preparation, and combines analysis of two drug classes into a single assay.

Analytes included in this method are: 6-Monoacetyl Morphine (6-MAM), Codeine, Morphine, Oxycodone, Hydrocodone, Hydromorphone, Desalkylflurazepam, Alprazolam, α-

Hydroxyalprazolam, Diazepam, Nordiazepam, Lorazepam, Oxazepam, Temazepam, Triazolam, 7-Aminoclonazepam, and Clonazepam. Deuterated analogs of each analyte were used as internal standards.

Urine samples were hydrolyzed, centrifuged for 2 minutes and diluted 1:5 with LC mobile phase. LC/MS/MS analysis was performed on a Shimadzu Prominence LC stack interfaced to an Applied Biosystems hybrid triple quadrupole/linear ion trap mass spectrometer. Injection-to-injection analytical run time was 6.5 minutes. Two MRM transitions per analyte were monitored and one transition per internal standard. The Scheduled MRM™ algorithm was use for optimal method performance for this multi-analyte method.

Results showed that all analytes were successfully detected in the 6.5 minute run time utilized. The LLOQs for most analytes was around ≤5 ng/mL and all analytes had an LLOQ ≤50 ng/mL. Precision and accuracy were both within 10% except at or near the LLOQ, where both precision and accuracy were within 15%. The linear dynamic range was at least three orders of magnitude for all analytes.

An LC/MS/MS method was developed to quickly analyze common benzodiazepines and opiates in urine. The minimal sample preparation, combined with short LC/MS/MS run time drastically decreased sample turnaround time and increased throughput without compromising sensitivity or selectivity. Additionally, the ability to combine two assays into one quick LC/MS/MS run further decreased analysis times and costs.

**Opiates, Benzodiazepines, LC/MS/MS**

## K12 A New Approach in Forensic Toxicology: Dimercaptosuccinic Acid (DMSA) Provocated Urine Potential Toxic Metal Test by Inductively Coupled Plasma - Mass Spectrometer (ICP - MS)

*Selda Mercan, MS, T. Mehmet Karayel, BS, Zeynep Turkmen, MS, and Salih Cengiz, PhD*, Istanbul University, Institute of Forensic Sciences, Istanbul Universitesi, Adli Tip Enstitusu, Cerrahpasa Kampusu, PK.10, 34303, Istanbul, 34303, TURKEY*

After attending this presentation, attendees will understand the application of dimercaptosuccinic acid (DMSA) as a heavy metal provocateur in forensic toxicology and learn the analysis of DMSA provocated urine with Inductively Coupled Plasma Mass Spectrometer (ICP-MS) in cases of chronic heavy metal intoxication.

This presentation will impact the forensic community by serving DMSA provocated urine toxic analysis as a new approach in forensic, environmental, and workplace toxicology.

DMSA is one of the agent used as a chelator in treatment of cases of acute heavy metal intoxication. There is a notable increase between the results of ICP-MS analysis of urine samples with and without DMSA provocation in the cases of heavy metal intoxication. Urine samples with DMSA provocation had high toxic metal concentration (Hg, Pb, Ni, Ar, Sn, Sb) considering to urine samples without DMSA. Also the observation of the highest potential toxic element limits in DMSA provocated urine samples of healthy individuals was notable. After this presentation, possibility and usefulness of this new application other than authentic methods in the determination of toxic metal limits will be highlighted.

Potential toxic metal analysis can be done by investigation of provocated urine samples taken from healthy individuals. Although some other provocation agents are present, DMSA should be chosen, since it is preferred also for children. However, the administered DMSA amount should be evaluated by a clinician based on her/his health condition and physical situation such as age, height, weight, etc.

The evaluation can be done among the healthy individuals exposed toxic metals for any reason whatsoever (chronic intoxication, workplace toxicity, environmental exposure, illness, etc.). Individuals may be classified according to their living regions and appropriate precautions may be taken by determination of workplace exposure limits.

A total of 36 trace and potential toxic elements were analyzed from 10 urine samples after appropriate sampling by using elemental analysis method. ICP-MS, which has wide range usage recently as a sensitive and quick and well interpretted method, was preformed in this study.

The toxic element limits between the DMSA provocated and non provocated urine samples of children and healthy individuals was compared. On the basis of data obtained from results of this comparison, authors are in the opinion of using DMSA, which representing possible new application in the field of forensic science, environmental toxicology and workplace toxicology.

**DMSA, Toxic Metal Analysis, ICP-MS**

## K13 The Second Seven Years of the FAA's Postmortem Forensic Toxicology Proficiency-Testing Program

*Patrick S. Cardona, BA\*, Federal Aviation Administration, AAM-610, CAMI Building, 6500 South MacArthur Boulevard, Oklahoma City, OK 3169-6901*

After attending this presentation, attendees will have an awareness of the FAA postmortem forensic toxicology proficiency testing program its impact to the PT participants during its second seven years of existence.

This presentation will impact the forensic community by informing attendees of the positive benefits of accreditation and quality control/quality assurance for those who participate in the program.

Attendees will be acquainted with the analytical findings of survey samples of the Federal Aviation Administration's (FAA's) postmortem forensic toxicology proficiency-testing (PT) program.

For aircraft accident investigations, samples from pilot fatalities are analyzed at the FAA's Civil Aerospace Medical Institute (CAMI) for the presence of combustion gases, alcohols/volatiles, and drugs. Throughout this forensic toxicological process, a high degree of quality control/quality assurance (QC/QA) is maintained, and quality improvement is continuously pursued. Under this philosophy, CAMI started a quarterly forensic toxicology PT program in July 1991 for the analysis of postmortem specimens. In continuation of the first seven years of the CAMI PT findings reported earlier, PT findings of the next seven years (July 1998–April 2005) are summarized herein. During this period, 28 PT challenge survey samples (12 urine, 9 blood, and 7 tissue homogenate) with/without alcohols/volatiles, drugs, drug metabolites, and/or putrefactive amine(s) were submitted to an average of 31 participating laboratories, of which an average of 25 participants returned their result sheets—that is, 53–96% (mean = 82%). The number of respondents was dependent upon the complexity of the sample matrix, the number and types of analytes in the sample, and the associated analytical chemistry/toxicology. For example, ethanol/methanol/volatiles in urine were correctly quantitated by a higher number of participants than those for amphetamine/methamphetamine and cannabinoid levels in blood and tissues. Methods employed ranged from immunoassays to gas chromatography-mass spectrometry/high performance liquid chromatography. Analytes in survey samples were correctly identified and quantitated by a large number of participants, but some false positives of concern were reported as some of them were abused drugs. Some of the false positives would have been avoided by not reporting those drugs solely based upon qualitative analyses. Their presence should have been confirmed, authenticated, and, if possible, quantitated by other analytical methods, which should have been based upon different analytical principles than those used during qualitative analyses. It is anticipated that the FAA's PT program would continue to serve as a tool to effectively allow its own toxicology laboratory and other participating laboratories for professional and technical maintenance and advancement on a voluntary, interlaboratory, and self-evaluative basis. Furthermore, this PT program will continue to provide service to the forensic toxicology scientific community through this important part of the QC/QA for the laboratory accreditation to withstand professional and judicial scrutiny of analytical results.

This presentation will summarize the PT results of the participating laboratories in the field of forensic toxicology. By understanding those survey results and applying related necessary procedures, the overall performance of a laboratory should improve. Participation of laboratories in external PT programs is a realistic approach for continuous quality improvement.

**Toxicology, Proficiency-Testing, Quality Improvement**

## K14 Quantitative Determination of Ethylene Glycol Using Capillary Gas Chromatography by Direct Specimen Injection

*Trista M. Haupt, BS\*, Emily Lemieux, BS, and Kenneth E. Ferslew, PhD, Section of Toxicology, East Tennessee State University, PO Box 70422, Johnson City, TN 37614-0422*

The goal of this presentation is to demonstrate how this method is useful in forensic and clinical cases to determine ethylene glycol concentrations by gas chromatography.

This presentation will impact the forensic science community with a more efficient and accurate method for quantification of ethylene glycol in blood or serum.

Ethylene glycol (EG) can be accidently ingested or sometimes abused by alcoholics for intoxication when no other form of alcohol is available; in either situation, untreated poisonings from overdose can be fatal. The consequences of consuming EG range from central nervous system depression to anionic acidosis and eventually death. Pathologists commonly discover calcium oxalate crystals while reviewing EG poisonings deposited in the brain, lungs, kidneys, and heart. EG blood concentration > 20 mg/dL should receive medical treatment; > 50 mg/dL are usually associated with severe intoxication; and > 200 mg/dL have been lethal. An approximate lethal oral dose of 95% EG is 1.5 mL/kg. The goal of the present work is to develop an effective method to analytically measure EG concentrations in biological fluids. Gas chromatographic methodology was performed on a gas chromatograph (GC) equipped with an auto sampler using a 5 µL syringe for a 1 µL injection; 4mm internal diameter splitter liner (@ 235°C); a 30 meter, 0.32 mm internal diameter, 1.80 µm film thickness novel stationary phase column and a 10 meter guard column (using a time temperature program of 100-140°C at 7.0°C/min, then 140-170°C at 40 °C/min); a flame ionization detector (@ 240°C); and helium as a carrier gas (@ 1.5 mL/min). EG and 1,2 propylene glycol (internal standard) separate at retention times of 3.58 and 3.77 minutes, respectively. Time temperature programming maximizes oven temperature to ensure all biological material is eliminated following injection. Computer software was used to analyze chromatograms for peak identification and quantitation. Acetone, methanol, ethanol, and isopropanol do not interfere with the chromatography of the glycols. The method is linear over a range of 20

to 200 mg/dL. Samples above the linear range are diluted appropriately with deionized water to fit within the standard curve.

Between-day and within-day replication of three controls (37.5, 75, and 150 mg/dL) were analyzed to test the reproducibility and accuracy of the method. Results of within-day replication (n=6) of the controls were (mean concentration ± SE, coefficient of variation): 37.8 ± 0.166; 1.08%; 77.2 ± 0.307, 0.976%; and 157.2 ± 1.19, 1.86%. Likewise, results of between-day replication of controls (n=6) revealed (mean concentration ±SE, coefficient of variation): 37.6 ± 0.211, 1.37%; 76.5±0.428, 1.37%; and 153 ± 2.29, 3.67%. Determination of the limit of detection was determined by serial dilution to be 1 mg/dL. The limit of quantitation for the method yielded a significant concentration of 5 mg/dL. The usefulness of this method was confirmed by application to clinical specimens. Case in point, a 39-year-old male was admitted to the hospital after consuming EG. EG blood concentrations were determined using this method ranging from 382 to 67 mg/dL. EG was removed from the patient's circulation by hemodialysis to an undetectable concentration over a four day period and physicians were able to stop treatment. GC of biological fluids by direct injection onto a capillary column has proven to be an effective, sensitive, and accurate method for determining EG blood concentrations. Distinct advantages of direct injection, capillary GC over other methodologies is that it is rapid, does not require any special specimen preparation and only requires a minimum of 10 µL of specimen. This method is useful in forensic and clinical cases to determine EG concentrations.

**Ethylene Glycol, Gas Chromatography, Capillary**

## K15 A Simple Liquid - Liquid Extraction of Carisoprodol and the Metabolite Meprobamate From Suspected Blood and Urine DUI Specimens for GC/MS Analysis

*Jamie L. Jouett, BS\*, Sam Houston State University, 13 FM 1696 East Huntsville, TX 77320*

After attending this presentation, attendees will understand a modified and improved method for analyzing carisoprodol and the metabolite meprobamate in blood and urine specimens. The goal of this presentation is to demonstrate a quick, clean, and effective liquid-liquid extraction method to detect the presence of carisoprodol (Soma®) and the metabolite meprobamate in blood/urine specimens at levels above, below, or at therapeutic concentrations, in turn, providing supportive analytical data for the assessment of suspected DUI cases. Literature data indicates severe driving impairment and intoxication when the combination of the two drugs exceeded 10 mg/L, a level that is still within normal therapeutic range.[1] Ultimately, this extraction method will prove a number of advantages compared to previously reviewed extraction methods.[2]

This presentation will impact the forensic community by demonstrating a clean and effective gas chromatography/mass spectroscopy (GC/MS) based and validated method for detection of carisoprodol (Soma®) and the metabolite meprobamate in suspected driving under the influence (DUI) specimens.

Carisoprodol is a commonly prescribed muscle relaxant that has not been classified as a controlled substance. The Brazoria County Crime Laboratory has observed that in suspected impaired drivers, the frequency of blood/urine specimens testing positive for carisoprodol and the metabolite meprobamate has increased over the past few years. This data reflects the obvious need for a simple, validated extraction method to confirm carisoprodol and the meprobamate in suspected impaired drivers.

To demonstrate a quick, clean, and effective liquid-liquid extraction method to detect the presence of carisoprodol and the metabolite meprobamate in blood/urine specimens at levels above, below, or at therapeutic concentrations, in turn, providing supportive analytical data for the assessment of suspected DUI cases. Literature data indicates severe driving impairment and intoxication when the combination of the two drugs exceeded 10 mg/L, a level that is still within normal therapeutic range.[1] Ultimately, this extraction method will prove a number of advantages compared to previously reviewed extraction methods.[2]

In this method, samples were prepared by adding buffer, barbital (internal standard) and chloroform to 250 uL of specimen. Barbital is the recommended internal standard due to the fact that it does not co-elute with targeted drugs of interest and is compatible with systems other than GC/MS, such as flame ionization detection (FID). The extraction efficiency and linearity of carisoprodol and meprobamate were analyzed at levels consistent with DUI blood/urine by comparing different buffer systems and adjusting pH levels. Buffer systems and pH adjustments evaluated were 0.1 M acetate buffer pH 4.5 and 0.1 M acetate buffer pH 4.5 saturated with NaCl, 1.0 M acetate buffer pH 4.5, and 0.1 N HCl. A five point calibration curve including 4mg/L, 10mg/L, 30mg/L, 40mg/L, and 60mg/L was utilized to determine linearity. After mixing, the chloroform was pipetted into a clean test tube and evaporated to dryness under nitrogen. The residue was reconstituted with 120uL of ethyl acetate and analyzed using an Agilent Technologies 6890 GC coupled to a 5975 MSD in electron sensitive-selective ion monitoring (EI-SIM) mode for quantitative analysis. GC injection conditions were evaluated under splitless, split, and pulsed-split modes.

The evaluation of this extraction method was based on precision, cleanliness, and chromatographic data. The 0.1 M HCL acidification results in a dirtier extract and tends to build residue in the injector port faster than the acetate buffering systems. Moreover, both 1.0 M acetate buffer and 0.1 M acetate buffer pH 4.5 saturated with NaCl demonstrate a more compacted protein layer between the aqueous and organic layers, resulting in a cleaner extraction. A cleaner extract reduces residue build up and drug decomposition in the GC injector port; thereby, minimizing routine instrument maintenance. However, the 1.0 M acetate buffer pH 4.5 assures the pH stability of blood and urine during extraction and is therefore the preferred buffering system. Chromatographic data were evaluated by comparing split, splitless, and pulsed-split modes. Split and pulsed-split modes offer improved peak symmetry and less column overload. In addition, calibration curves were linear from 4-60 mg/L with $R^2$ values of 0.995 for carisoprodol and 0.999 for meprobamate. The extraction efficiencies were 48% (barbital), 69% (carisoprodol), and 71% (meprobamate). Thus, 1.0 M acetate buffer pH 4.5 is the optimal buffering system to provide clean extracts with consistent recoveries.

This extraction procedure provides a rapid, clean, and effective method suitable for detecting carisoprodol and meprobamate with the intended purpose of providing analytical data to determine drug concentrations in suspected DUI cases.

**References:**
1. Journal of Forensic Science 2000; 45(3):619-23
2. Journal of Analytical Toxicology 2006; 30(5):302-5

**Carisoprodol, Meprobamate, GC/MS**

## K16 Issues Pertaining to the Analysis of Buprenorphine and its Metabolites by GC-MS

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After attending this presentation, attendees will better understand the low-cost and widely available GC-MS technology can be effectively applied to the analysis of buprenorphine (B) and its metabolites in urine specimens.

This presentation will impact the forensic community by reporting the following issues pertaining to the analysis of B and its metabolites by GC-MS: (a) selection of extraction methods for the determination of free and total B and norbuprenorphine (NB); (b) effectiveness of hydrolysis, derivatization, and internal standard; and (c) deriving the contents of the glucuronides based on the free and total concentrations of B and NB observed from a two-step analytical protocol.

"Substitution therapy" and the use of B as an agent for treating heroin addiction continue to gain acceptance and have recently been implemented in Taiwan. Mature and widely utilized GC-MS technology can complement the low-cost and highly sensitive immunoassay (IA) approach to facilitate the implementation of analytical tasks supporting compliance monitoring and pharmacokinetic/pharmacodynamic studies. Issues critical to GC-MS analysis of B and NB (free and as glucuronides), including extraction, hydrolysis, derivatization, and internal standard, are studied, followed by comparing the resulting data against those derived from IA and liquid chromatography-tandem mass spectrometry methods. Commercial solid-phase extraction devices, highly effective for recovering all metabolites, may not be suitable for the analysis of free B and NB; acetyl-derivatization products exhibit the most favorable chromatographic, ion intensity, and cross-contribution characteristics for GC-MS analysis; B-d$_4$ can effectively serve as the single internal standard for the quantitations of both B and NB. The 2-aliquot GC-MS protocol hereby developed is proven effective for the analysis of free B and NB and their glucuronides.

**Buprenorphine, Glucuronide, GC-MS**

## K17 GC/MS Method Development for the Quantitation of Quetiapine in Various Biological Specimens

*Jennifer M. Hogue, BA\*, 2501 Lake Road, #126, Huntsville, TX 77340, and Laureen Marinetti, PhD, Montgomery County Coroner's Office, Miami Valley Regional Crime Lab, 361 West Third Street, Dayton, OH 45402*

After attending this presentation, attendees will be aware of a GC-MS method that can be used to detect and quantitate the presence of quetiapine in biological specimens using chemicals and instrumentation that is widely available in most laboratories.

This presentation will impact the forensic community by providing a new method for the detection of quetiapine.

Quetiapine ($C_{21}H_{25}N_3O_2S$) is classified as a dibenzothiazepine derivative and is used clinically as an antipsychotic for the treatment of schizophrenia and bipolar disorder. In the body, quetiapine acts as an antagonist, targeting the serotonin and dopamine receptors. Quetiapine is metabolized in the liver, with 73% eliminated through the urine. Less

than 1% of the parent drug is eliminated unchanged. Quetiapine is known to be 83% plasma protein bound and have a volume of distribution of $10 \pm 4$ L/kg. It is administered orally as a fumarate salt in 25mg, 100mg, 200mg, 300mg, and 400mg tablets. The fumarate salt is comprised of two quetiapine molecules per one fumarate molecule (MW = 883.1). The drug is structurally similar to the antipsychotic drug clozapine.

The Montgomery County Coroner's Office (MCCO) encountered quetiapine in 47 cases in 2007. Incidents of quetiapine in casework are increasing and as MCCO did not possess a method for the quantitation of quetiapine, specimens had to be analyzed by an outside laboratory. A study was completed identifying an extraction and instrumental procedure for the detection and quantitation of quetiapine in order to diminish the cost of outside testing. The postmortem specimens analyzed were blood, brain, liver, cerebral spinal fluid, bile, vitreous fluid, and urine. The internal standard was Smith Kline French-525A (SKF-525A). Calibrators were prepared from a quetiapine stock standard solution at concentrations of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0μg/mL. Liquid and powder forms of quetiapine were prepared for controls. The analysis was completed by following an in-house liquid-liquid extraction for basic drugs. Quetiapine was extracted with hexane/isoamyl alcohol (99/1). Back extraction was completed by the addition of hydrochloric acid. The drug was re-extracted into methylene chloride, which was then evaporated to dryness. Derivatization was completed by reconstituting with BSTFA + 1% TMCS and heating for 20 minutes at 75ºC. One microliter was injected on an Agilent 5973 Series gas chromatograph mass spectrometer (GC-MS) with a DB-5MS (30m x 0.25mm x 0.25μm) column. The temperature program has an initial temperature of 100ºC with an increase of 20ºC per minute to a final temperature of 285ºC. Single ionization mode (SIM) was used with the quetiapine target quantitation ion 210 and the qualifier ions 239 and 321. The target quantitation ion for the internal standard was 86. The assay was linear from 0.01 - 2.0μg/mL.

Quetiapine was identified to have a retention time of 22.64 minutes. Linear regression analysis indicated an $R^2$ value of 0.9952 over the entire calibration range. The concentration range of quetiapine in twelve blood specimens was 0.16 – 1.75μg/mL. The postmortem distribution of quetiapine in all other specimens were as follows: brain 0.10-1.90μg/mL (6 cases), liver 0.14-1.69μg/mL (6 cases), cerebral spinal fluid 0.10-0.18μg/mL (3 cases), bile 0.10-0.64μg/mL (3 cases), vitreous fluid 0.12-1.42μg/mL (4 cases), and urine 0.01-0.77μg/mL (7 cases). The completion of this study identifies a method that MCCO can utilize to detect and quantitate quetiapine.

**Quetiapine, Postmortem Toxicology, Gas Chromatograph Mass Spectrometry**

## K18 Postmortem Analysis of Buprenorphine/ Norbuprenorphine From Whole Blood by GC/MS

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After attending this presentation, attendees will understand the general principles of burprenorphine, the prevalence and use of the drug in society, and the importance of analyzing for it at the Los Angeles County Department of Coroner.

The presentation will impact the forensic community by providing information on how to extract Buprenorphine and Norbuprenorphine from postmortem specimens with detection by gas chromatography/mass spectrometry (GC/MS).

Buprenorphine, is a semi-synthetic chemical derivative of thebaine

which is used to relieve moderate to severe pain. As of 2002, the FDA approved the use of buprenorphine tablets for treatment of opioid addiction. The number of cases seen at the Los Angeles County Department of Coroner involving buprenorphine has slowly increased in the past few years because of their use in addiction clinics. The current literature describes methods for buprenorphine detection in various matrices such as hair, urine, and whole blood by LC/MS/MS; however, most do not require a comprehensive sample preparation necessary for GC/MS detection. Given the fact that most forensic toxicology laboratories are equipped with the GC/MS rather than the LC/MS/MS the object of this study was to develop and validate a method for the extraction of buprenorphine and its active metabolite, norbuprenorphine from postmortem blood with detection by GC/MS. The analysis consisted of a protein precipitation with acetonitrile, solid phase extraction, and silylation derivitazation with MSTFA. Quantitation was performed with the use of deuterated internal standards, d4-Buprenorphine and d3-Norbuprenorpine and the instrument was operated in the selected ion monitor (SIM) mode with the following ions:

| d4-Buprenorphine | Buprenorphine | d3-Norbuprenorphine | Norbuprenorphine |
|---|---|---|---|
| 454 * | 450 * | 527 * | 524 * |
| 486 | 482 | 509 | 506 |
| 510 | 506 | 528 | 525 |

* Quantitaion ion

Linearity was achieved over a concentration range of 2.0 – 25 ng/ml for both drugs supplemented in porcine whole blood with a correlation coefficient exceeding 0.99. The percent recovery of buprenorphine (83%) and norbuprenorphine (68%) was determined at three concentrations (2.0, 5.0, and 10 ng/ml) over four separate days. Limit of quantitation was 2.0 ng/ml and the upper limit of linearity (beyond 25 ng/ml) was not explored as casework would be repeated at a dilution to be within the curve. The intra-assay reproducibility (n=4) was determined for buprenorphine 2.0 ng/ml (CV 11.53%), 5.0 ng/ml (CV 7.86%), 10 ng/ml (CV 4.81%) and norbuprenorphine 2.0 ng/ml (CV 11.78%), 5.0 ng/ml (CV 7.93%), and 10 ng/ml (CV 4.73%). The inter-assay reproducibility (n=12) was determined for buprenorphine 2.0 ng/ml (CV 8.30%), 5.0 ng/ml (CV 7.05%), and 10 ng/ml (CV 4.37%) and norbuprenorphine 2.0 ng/ml (CV 9.47%), 5.0 ng/ml (CV 7.74%), and 10 ng/ml (CV 4.05%). The method was determined to be free from matrix interferences (liver, bile, and urine) by the supplementation of buprenorphine and norbuprenorphine, in duplicate, at 10 ng/ml. Quantitation of both drugs were not affected by any matrix when compared with a blood calibration curve; however, the recovery of norbuprenorphine was severely diminished in the liver specimen, whereas, all the others had no effect. Lastly, the method was successfully verified by the comparison of three different external controls as well as casework that had previously been outsourced.

Buprenorphine is increasing in popularity both on the streets as well as being used in addiction clinics. Therefore, the analysis of buprenorphine and metabolite needs to be a common practice amongst postmortem toxicology laboratories.

**Buprenorphine, Analysis, Postmortem**

# K19  A 5-Year Stability Study on Phencyclidine and Zolpidem in Postmortem Blood Samples

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The goal of this presentation is to evaluate the stability for both phencyclidine and zolpidem over a 5-year time period in postmortem blood samples.

This presentation will impact the forensic community by demonstrating the effects of storage conditions and time on postmortem blood samples containing either phencyclidine or zolpidem and contributing additional knowledge to the proper interpretation of reanalyzed samples containing these drugs.

Phencyclidine, or PCP, is a dissociative anesthetic but exhibits stimulant, depressant, hallucinogenic, and analgesic properties. Phencyclidine is an illicit, Schedule II drug. Zolpidem is a sedative-hypnotic, Schedule IV drug, classified as a derivative of the imidazopyridines. Zolpidem is available by prescription and is used in the treatment of short-term insomnia. Due to the abuse of phencyclidine and the increase in popularity and overdose potential of zolpidem, it is essential to show how stable these drugs are in postmortem samples.

The stability for drugs in postmortem samples is extremely critical in establishing the validity of scientific results. Stability for the analyzed drug should be considered in order to justify the precision of the analytical method and the reliability of the results. Factors that may influence drug stability in stored samples include: storage temperature, storage time, addition of preservatives, and initial condition of the collected sample. Storage conditions may vary depending on the analyte of interest. This study will test the research hypothesis of whether drug concentrations for either phencyclidine or zolpidem remain stable, when samples are frozen at –20ºC for up to five years. The quantitative examination for stability of 23 positive phencyclidine cases and 26 positive zolpidem cases from the Maricopa County Office of the Medical Examiner (OCME) is presented here.

This study re-analyzes postmortem blood samples quantitatively for any changes in concentration of phencyclidine and zolpidem, over 5 years. The postmortem blood samples were collected at autopsy, preserved with sodium fluoride, and stored at 4ºC until initially analyzed. After the analysis the samples were stored frozen at –20ºC until the samples were reanalyzed for this study. The methods of quantitation used in the re-analysis study are the same methods used when initially quantitated. For phencyclidine (n=23) liquid-liquid extraction is used followed by quantitation by gas chromatography/mass spectrometry (GC/MS). For zolpidem (n=26) liquid-liquid extraction is used followed by quantitation by gas chromatography with a nitrogen-phosphorous detector (GC-NPD).

The results obtained for phencyclidine show a tendency for concentrations to decrease over a period of 5 years. Table 1 shows the initial and final concentration ranges obtained (reported to two significant figures), along with the average decreases observed for samples stored for 5 years.

Table 1. Phencyclidine Concentration Changes

| Storage Time (Since Collection) | N | Initial Concentration Range (mg/L) | Final Concentration Range (mg/L) | Percent Average Decrease |
|---|---|---|---|---|
| + 1 year | 8 | (0.01 – 0.22) | (<0.01 - 0.20) | 18.9% |
| + 2 years | 5 | (0.03 – 0.11) | (0.02 – 0.10) | 8.6% |
| + 3 years | 3 | (0.09 – 0.23) | (0.07 – 0.18) | 21.5% |
| + 4 years | 3 | (0.02 – 0.14) | (0.01 – 0.13) | 18.8% |
| + 5 years | 4 | (0.04 – 0.73) | (0.03 – 0.48) | 20.3% |

For the phencyclidine cases (n=23), there were 12 cases showing a decrease of 10% or more, of which 9 of these cases had a decrease of 20% or more. The results indicate that phencyclidine remains sufficiently stable to be detected within 5 years of storage at 4ºC, then –20ºC. However, there is a significant decrease in concentration after 3 years of storage at 4ºC, then –20ºC.

The results obtained for zolpidem show a tendency for concentrations to both increase and decrease over a period of 5 years. Table 2 shows the initial and final concentration ranges obtained (reported to two significant figures), along with the average decreases and increases observed for samples stored for 5 years.

Table 2. Zolpidem Concentration Changes

| Storage Time (Since Collection) | N | Initial Concentration Range (mg/L) | Final Concentration Range (mg/L) | Percent Average Decrease | Percent Average Increase |
|---|---|---|---|---|---|
| + 1 year | 12 | (0.06 – 34.44) | (0.07 – 34.60) | 24.1% (n=5) | 12.6% (n=7) |
| + 2 years | 8 | (0.15 – 0.97) | (0.15 – 0.80) | 16.1% (n=3) | 10.1% (n=5) |
| + 3 years | 2 | (0.29 – 3.39) | (0.20 – 3.46) | 29.6% (n=1) | 2.3% (n=1) |
| + 4 years | 2 | (1.20 – 8.42) | (0.73 – 5.20) | 38.6% (n=2) | - |
| + 5 years | 2 | (0.10 – 0.28) | (0.09 – 0.23) | 9.5% (n=2) | - |

For the zolpidem cases (n=26), there were 11 cases showing a decrease of 10% or more, of which 4 of these cases had a decrease of 20% or more. The results also show that for 7 cases the concentrations increased 10% or more over time, of which 3 of these cases had a 20% or more increase. The results indicate that zolpidem remains sufficiently stable to be detected within 5 years of storage at 4ºC, then –20ºC. However, there is a significant decrease in concentration within 1-year of storage at 4ºC, then –20ºC.

**Stability, Phencyclidine, Zolpidem**

# K20 Method Development for the Analysis of Non-Traditional Drugs Used to Facilitate Sexual Assaults

*Jennifer L. Greaux, BS*, and Bruce R. McCord, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199*

After attending this presentation, attendees will become aware of a wide array of "non-traditional" drugs which have the potential to be used to facilitate sexual assaults. In addition, attendees will gain insight into the use of capillary electrophoresis (CE) for drug analysis and the advantages and disadvantages of using such a technique when coupled to a UV detector and an electro spray ionization time-of-flight mass spectrometer (ESI-TOF-MS).

This presentation will impact the forensic community by providing a more efficient technique for drug analysis and introducing new methodology for analyzing "non-traditional" drugs which have the potential to be used to facilitate sexual assaults.

The term drug-facilitated sexual assault (DFSA) has been assigned to cases where a drug(s) has been used to incapacitate an individual so that he/she is unable to consent to sexual activity.

The overall purpose of this project was to develop and optimize methods for the analysis of drugs which may be found in blood and urine specimens from sexual assault cases. It was desirable that these methods also provide accurate identification and confirmation when compared to standards. Drug standards have been prepared at various concentrations in buffer and deionized water and separated using CE-UV and CE-MS. These mixtures were comprised mainly of drugs belonging to the following classes: anticholinergic, anticonvulsant, antidepressants, antihistamines, antihypertensive, cough suppressants, and muscle relaxants. The compounds selected have been identified as candidates for DFSA because they may cause sedation, amnesia, and lower an individual's ability to resist a sexual assault.

Some of the problems surrounding sexual assault samples are that there is a limited time window for detection and that the drugs may have widely varying chemical properties and may be present in very low concentrations. Therefore, a technique is required that is fast, efficient, and very sensitive for DFSA samples. It is proposed that CE coupled to MS may be a useful technique to analyze these compounds due to its high resolution and wide range of sample detection capabilities. In addition, the application of time of flight mass spectrometry greatly improves the ability to detect and identify unknown analytes. Due to its high (3ppm) resolution, the time of flight system permits infusion of samples prior to separation as a quick and efficient prescreening tool.

Optimization of developed methods was performed by altering parameters such as buffer pH and concentration, voltage, and sample injection. Additionally, the effects of adding organic modifiers and a water plug were examined. Phosphate buffer at low pH was used as the run buffer as it will cause the drugs to remain charged and suppress the electroosmotic flow to allow sufficient time for separation. The limits of detection and reproducibility of results were also evaluated to determine the relevance of this study to "real-life" samples.

The analysis of various drug mixtures will be detailed to show that capillary electrophoresis is an efficient and reliable technique for drug detection of sexual assault samples. Such a technique can then be used to aid authorities in prosecuting criminals accused of sexual assault in a quick but efficient manner.

**Capillary Electrophoresis, DFSA, Method Development**

# K21 Screening of Anabolic Steroids in Suspected DUI Drivers in Miami - Dade Florida Using ELISA Kits

*Lisa J. Reidy, PhD*, Bernard W. Steele, MD, and H. Chip Walls, BS, Forensic Toxicology Laboratory, University of Miami, Department of Pathology, 12500 Southwest 152nd Street, Building B, Miami, FL 33177*

After attending this presentation, attendees will better understand the possible role that steroids may play in suspected DUI drivers. The goal of this presentation is to suggest a reliable screening method for the steroids boldenone and stanozole in biological samples.

This presentation will impact the forensic community by providing information on a reliable methodology for the screening of steroids in urine and blood and establishing the incidence of steroid abuse in the suspected DUI community of Miami-Dade County. This information is important to establish possible drug abuse patterns in our communities and help identify possible causation factors for suspected drug impaired driving cases.

Anabolic steroids such as boldenone and stanozolol are compounds related to testosterone. Steroids are reported as being abused by professional athletes to increase strength and muscle mass; however, there are reports of abuse amongst the general population. It is understood that high doses of anabolic steroids can cause aggressive behavior, insomnia, and irritability. Anabolic steroids have been also reported to cause other behavioral effects, including euphoria, increased energy, sexual arousal, mood swings, distractibility, forgetfulness, and confusion. These reported side effects may have an effect on driving skills and therefore may be compounds of interest in suspected DUI drivers.

The purpose of this study was to evaluate the occurrence, if any, of boldenone and stanozolol in suspected DUI drivers in Miami-Dade County. If a sample is recorded as positive then the "Drug Recognition Expert" evaluation was examined to correlate symptoms with drug use.

Blood and urine samples were submitted to the forensic toxicology

lab for drug and alcohol screening. Boldenon and stanozolol were screened for in blood and urine by ELISA kits for all samples received in 2008. In addition, all blood and urine samples over the past 5 years that were negative in routine drug screens were analyzed. If positive, steroids were qualified and quantified in blood/urine by GC-MS. Case histories, including the DRE evaluation were collected and positive results were evaluated using this information.

Providing information on possible steroid abuse may explain some behavior and impairment seen in suspected DUI drivers when all other toxicological screening is negative. This information is important to determine potential drug abuse in our community and help identify possible causation factors for suspected drug impaired driving cases.

**Anabolic Steroids, ELISA, Driving**

## K22 Rapid Inline Derivatization of Primary and Secondary Amine Containing Drugs Using NBD-F and CE-LIF

*Britt E. Turnquest, BSc\*, Florida International University, 11200 Southwest 8th Street, Miami, FL 33199; and Bruce R. McCord, PhD, Department of Chemistry, Florida International University, University Park, Miami, FL 33199*

After attending this presentation, attendees will be able to understand the mechanism by which drugs containing primary and secondary amine groups are derivatized on — capillary using NBD — F for the purpose of detection using capillary electrophoresis with laser-induced fluorescence.

This presentation will impact the forensic community by explaining how this method allows for the sensitive and rapid detection of drugs in bodily fluids for the purpose trace analysis and general drug screening.

Capillary electrophoresis has become an increasingly common analytical method in forensics due to its flexibility and the wide variety of detection systems which can be used. One particularly useful method of detection in CE is laser-induced fluorescence, LIF. The application of LIF permits highly sensitive detection of compounds using CE in spite of the narrow pathlength inherent in the procedure. However, the number of compounds in which fluorescence occurs naturally are few and in order for most compounds to fluoresce, derivatization is necessary.

There are a variety of fluorescent dyes which can be coupled to primary and secondary amines. Derivatization can take place through reactions with dyes linked to reactive groups such as isothiocyanates, succinamidyl esters, and other amine reactive groups. These derivatizations can be performed before the analyte enters the capillary, while it is on the capillary during the separation or after the separation has been completed, post-capillary. On-capillary derivatization is not used as commonly as pre-capillary or post-capillary derivatization due to difficulties in reproducibility and optimization of the derivative yield. If these issues could be overcome it would greatly increase the throughput of analyses given and permit the use of inline, microfluidic techniques.

A feature common to many drugs of abuse are primary, secondary, or tertiary amine moieties. NBD-F is a non-fluorescent compound which reacts to primary and secondary amines by losing the fluorine attached to the benzene ring and joining to the analyte at the nitrogen which loses a hydrogen atom. The resulting derivative is strongly fluorescent and has an emission wavelength around 530 nm. Given that prior to derivatization NBD-F is not fluorescent, the excess reagent produces minimal interference with the analyte permitting sensitive and specific detection of the drug conjugates. The overall process permits a highly sensitive and rapid screen for drugs in body fluids.

This paper will discuss the development of in-line derivatization techniques for trace detection and screening of phenethylamines and other drugs of abuse.

**On-Capillary Derivatization, Phenethylamines, Capillary Electrophoresis**

## K23 Importance of Postmortem Adipose Tissue Analysis in an Olanzapine (Zyprexa) Suicide Case

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The goal of this presentation is to suggest the value of adipose tissue analysis for the identification of drug users is steadily gaining recognition. Adipose tissue analysis may be useful adjunct to conventional drug testing in toxicology. Specimens can be more easily obtained with less embarrassment and adipose tissue can provide a more accurate history of drug use. After attending this presentation, attendees will understand the importance of biological alternative matrices in postmortem toxicological analysis.

This presentation will impact the forensic community by demonstrating the utility of adipose tissue analysis in determining defensible cause of death to evaluate the pharmacological story. The objective of this presentation is to provide long-term information about an individual's drugs use, especially when the pharmacological history is difficult or impossible to obtain. A sensitive and specific GC/MS method for the determination of drugs in postmortem adipose tissue was used. The method combines acid extraction of analytes, alkalinization of the extract aqueous, purification on Extrelut NT columns and GC-MS analysis.

This case involves a 22-year old female who suffered from depression and was on benzodiazepines and antipsychotics: lorazepam, valproic acid, chlorpromazine, and sertraline. There was history of three previous attempted suicides. At the crime scene, a large number of antidepressants, antipsychotics and benzodiazepines packs (some of which were empty), and an empty olanzapine (Zyprexa) pack. Systematic toxicological analysis was performed on conventional biological samples for drug of abuse, alcohol, and other poisons. Urine immunochemical screening and GC/MS analysis detected all drugs prescribed, in therapeutic concentrations (lorazepam, valproic acid, chlorpromazine, and sertraline). Blood immunochemical screening and GC/MS analysis detected all drugs prescribed in therapeutic concentrations and a olanzapine (Zyprexa) concentration of 3.07µg/ml, greater than the therapeutic concentration range of 0.01- 0.05 µg/ml.

Toxicological analysis on adipose tissue confirmed the presence of all drugs prescribed (Lorazepam, valproic acid, chlorpromazine, and sertraline) and found at the crime scene, but did not reveal the olanzapine presence. The large presence of olanzapine, not prescribed drug, in the blood and not in adipose tissue is indicative of the olanzapine intake for suicide. Therefore, the death was ruled a suicide caused by olanzapine overdose. In conclusion, this study suggests the value of adipose tissue analysis for the identification of drug users and is steadily gaining recognition. Adipose tissue analysis may be useful adjunct to conventional drug testing in toxicology. Specimens can be more easily obtained with less embarrassment and adipose tissue can provide a more accurate history of drug use.

**Adipose Tissue Analysis, Olanzapine, Suicide**

## K24 Impact of Drugs and Alcohol on Manner of

## Death by Sex and Age Among Autopsy Cases Performed at the Upper East Tennessee Forensic Center in 2007

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After attending this presentation, attendees will understand of the impact of drugs and/or alcohol on the manner of death by sex and age among autopsies performed at the Upper East Tennessee Forensic Center in 2007.

This presentation will impact the forensic community by illustrating the increased impact of drugs and alcohol on the manner of death in a select region of Tennessee.

The Upper East Tennessee Forensic Center performs autopsies on the questionable and medicolegal deaths which occur in the eight counties of the First Tennessee Development District. Toxicological evaluations of specimens collected at autopsy are used to determine if drugs and/or alcohol are involved in determining the cause and manner of death. A descriptive database was established defining all parameters and data pertinent in each case (age, sex, cause/manner of death, and toxicological results). The purpose of this research was to determine descriptive statistics on the impact of drugs and/or alcohol by manner/cause of death, age, and sex in the autopsies performed in 2007. Specimens (blood, gastric contents, urine, vitreous humor, and bile) from the autopsies were analyzed for drugs and alcohol using multiple analytical toxicological procedures (colorimetric, TLC, immunochemistry, GC, GCMS, and LCMS). Toxicological results were compiled in an electronic database to allow for analysis and interpretation. Results indicate that out of 277 total cases, 66% were male, 34% were female, 85% were positive for drugs, 27% were positive for alcohol, 23% were positive for both drugs and alcohol, and 12% had neither drugs nor alcohol. Analysis of the distribution of cases positive for drugs, alcohol, and drugs/alcohol revealed that males had a greater percentage of cases involving alcohol alone as well as cases positive for drugs/alcohol than females. Acute drug overdoses accounted for 34% of total cases with no substantial sexual differentiation. Of the 94 acute overdose cases, 4 (»4%) were intentional (suicides) and 90 (»96%) were accidental. Autopsies were performed on all age groups (percentage of cases/ years of age): 5% <14, 4% between 15 and 19, 8% between 20 and 24, 14% between 25 and 34, 22% between 35 and 44, 16% between 45 and 49, 13% between 50 and 54, 10% between 55 and 64, 6% between 65 and 74, and 2% ³75 years of age. The distribution of positive drug cases closely mirrored the distribution of cases by age groups. Manner of death analysis revealed (of total cases) that 47% were accidental, 27% were natural, 15% were suicides 6% were homicides, and 5% were undetermined. No appreciable disparity in distribution of manner of death was found between the sexes. Analysis of the results of the toxicological evaluations revealed there were a large number of cases in which opiates (100), alcohol (75), benzodiazepines (110), sedatives (18), and/or stimulants (14) were identified. Review of these results leads to the conclusion that drugs and alcohol have a significant impact in the questionable and medicolegal deaths occurring in Upper East Tennessee.

**Drugs, Alcohol, Death**

## K25 Relationship Between Drug Levels and the Causes and Manners of Death in Methamphetamine Related Casualties: A Retrospective Study

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After attending this presentation, attendees will have knowledge of methamphetamine blood/urine levels distribution in the manners of death with a retrospective review cases for seventeen years.

This presentation will impact the forensic science community by utilization of the toxicological profiles in the determination of forensic parameters including the cause and manner of death, especially for methamphetamine related fatalities.

Methamphetamine (MAP), an illicit, stimulant drug, has resulted in serious social problems in Taiwan and other parts of the world. A pilot study was designed to determine whether toxicological profiles of decedents' body fluids can be used to implicate the status of mood at the moment of death. High blood/urine ratios can be associated with acute MAP use, shortly after MAP intake and a manic emotional status. In comparison, a low blood/urine ratio can be associated with chronic MAP use, after a longer period of time following MAP intake and a depressive emotional status. This retrospective review of 18,973 fatalities collected from Institute of Forensic Medicine in Taiwan from 1991 to 2007. MAP levels both in blood and urine that were greater than 0.02 mg/L and with positive impressions of the causes and manners of death were found in 212 cases. Distinct patterns of MAP levels were distinguished to be associated with manner or pattern of death.

Higher MAP concentrations were found in blood than in urine when death occurred shortly after an overdose of MAP that was linked either to accidental overdose ($3.24 \pm 0.73$ mg/L blood, $15.08 \pm 2.38$ mg/L urine and $22.07 \pm 4.22$ urine/blood ratio; n=88) or to intentional suicide ($12.81 \pm 5.30$ mg/L blood, $14.68 \pm 5.57$ mg/L urine, and $15.38 \pm 12.96$ urine/blood ratio; n=7). Lower MAP blood levels and urine/blood ratios were found in cases of accidental deaths ($0.31 \pm 0.06$ mg/L blood, $5.72 \pm 1.31$ mg/L urine and $34.86 \pm 9.81$ urine/blood ratio; n=30), and suicides not related to high MAP dose ($0.55 \pm 0.13$ mg/L blood, $10.35 \pm 2.75$ mg/L urine and $34.71 \pm 9.65$ urine/blood ratio; n=20), thus making a highly suspicion of influence of MAP mediated through depression and psychotic behaviors. Much higher MAP urine/blood ratios and lower MAP blood levels were found among casualties of natural causes ($0.40 \pm 0.09$ mg/L blood, $14.88 \pm 4.60$ mg/L urine, and $81.07 \pm 44.86$ urine/blood ratio; n=19) or homicidal causes ($1.26 \pm 0.19$ mg/L blood, $13.19 \pm 1.95$ mg/L urine, and $16.66 \pm 2.80$ urine/blood ratio; n=48), suggesting these were relatively unaffected by the lower blood level of MAP. Chronic MAP abusers appear to provoke violent behaviors resulting in the homicidal fatalities, and relationship to amphetamine (AMP)-like psychosis is postulated.

These results suggest that the toxicological profile of MAP concentrations in blood and urine can play a crucial role and are related better to patterns of death than manner of death. The findings may enable better utilization of the toxicological profiles in the determination of forensic parameters including the cause and manner of death in MAP

related fatalities.

**Methamphetamine, Manner of Death, Cause of Death**

## K26  An Unusual Case of Ethanol/Methanol Poisoning:  Or Was It?  The Million Dollar Question!

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After attending this presentation, attendees will be able to:  (1) Recognize common problems that can confound the correct interpretation of blood ethanol determinations, (2) Identify issues that can reduce the reliability of postmortem blood ethanol or methanol test results, and (3) Develop a set of questions which should be addressed regarding the integrity of any blood sample obtained from the living or deceased.

This presentation will impact the forensic community by explaining the confounding issues in the interpretation of blood ethanol test results can be reduced by obtaining the answers to several probative questions regarding person, site, and methods of blood drawing and storage.

Common problems can confound the correct interpretation of typical blood ethanol or drug tests, especially when the blood has been taken from a dead body.  The presented case is an example of some of the difficulties that can be encountered, and provides a set of questions which should be addressed about the integrity of any blood sample, but most importantly, a blood sample that has been obtained from a dead body.

A professional truck driver in Alaska was found dead in the cab of his truck after it was driven off the road and had rolled down a hill.  Three empty beer cans and a sandwich wrapper were found in the cab behind the driver.  Toxicology analyses from a certified laboratory reported a blood ethanol level of 0.086% (single value) and a blood methanol level of 0.15%.  If the driver was impaired at the time of the accident, worker's compensation would not pay death benefits to the decedent's wife and family.  However, if impairment was not proven, then the family would receive insurance and death benefits.

The insurance company claimed the driver had been impaired and retained a forensic pathologist who reviewed the laboratory tests and signed an affidavit stating that the decedent had ingested both ethanol and methanol (from Sterno) prior to death and had been impaired at the time the truck rolled down the hill.  This author was retained by the attorney for the widow and the family to investigate the circumstances of the ethanol and methanol blood analyses.

When the attorney called, I asked, "Who drew the blood sample?"  He responded, "I don't know."  I asked, "Where was the blood sample drawn, in the hospital?"  "No, it was drawn in a mortuary."  This answer provided the critical information to infer that the sample was unreliable, and, knowing that embalming fluid contained both formaldehyde and methanol, that the blood sample most likely was obtained after the body had been embalmed.

The rest of the case was easy.  Take the mortician's deposition, determine the body site from which the sample was obtained, discover the name of the company that supplied the chemicals used to embalm the body, and get copies of the Material Safety Data Sheets (MSDS) to ascertain the chemical composition of the embalming fluids.  The deposition also indicated that the mortician had not obtained a true blood sample.  Instead, he had found a small collection of blood-tinged fluid in the body cavity and had submitted that for testing labeled as "blood."  The MSDS for one of the two chemicals used to embalm the body stated that the product contained 20% methanol.

When blood samples are obtained in a hospital or by law enforcement, appropriate procedures are followed in order to assure the sterility and integrity of the sample in order to conform to existing standards and ensure the reliability of the results.  When blood samples are obtained from dead bodies, often the sterility of the sample cannot be assured, and contamination of the sample by bacteria can lead to the production of postmortem ethanol both *in situ* and *in vitro* (Zumwalt et al, 1982), which can lead to unreliable results.  Bacterial degradation and metabolism of endogenous substances like glucose (Clark et al, 1982), lactate (Bogusz et al, 1970), glycerol (from fat), and amino acids (Corry, 1977) also have been shown to produce ethanol in dead bodies.

While collecting and analyzing blood, other body fluids  like vitreous humor and urine (Levine et al, 1993) can be helpful in determining the source of the ethanol in blood, and asking a few simple questions about the acquisition of the blood sample also can be illuminating.  Such questions include: Who drew the blood sample?  Who performed the analysis?  What were the qualifications of the operator?  From what anatomical site was the blood sample obtained?  Was a preservative like sodium fluoride or sodium azide used?  Was an anticoagulant like EDTA, Ca oxalate, Ca citrate, or heparin used?  Was the blood "spun down" before storing?  Was there any hemolysis present in the sample?  Under what conditions was the sample stored?  How much time elapsed between sample drawing and analysis?  Which laboratory did the testing?  For ethanol, was the testing done with a non-specific Alcohol Dehydrogenase assay that measures NADH production or other screening-level test, or by gas chromatography?  What was the specificity of the test procedure?  What was the sensitivity of the test procedure?  Was the sample collected, transported and processed under a Chain of Custody?   Was a "test kit" used or a laboratory protocol?  If so, can you get a copy of the manufacturer's labeling or the laboratory's protocol?  Only questions provide answers.

**Integrity of a Blood Sample, Reliability of Test Results, Solving a Case**

## K27  Exsanguinating Hemorrhage From a Ruptured Gravid Uterus Resulting in Maternal and Near Full Term Infant Death Following Cocaine Abuse

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After attending this presentation, attendees will gain an understanding of some of the complications associated with heavy cocaine use during the late stages of pregnancy.  Specifically, this presentation addresses the postmortem analytical results of a mother and her in-utero fetus that died following uterine rupture after a period of cocaine use.  This information may be applicable to previous, current or future cases that involve similar circumstances, whether the case leads to the death of the fetus, mother, or both.

This presentation will impact the forensic community by providing pharmacokinetic data for a case in which limited data are currently available.  This presentation describes to the forensic sciences community a case that involves late term pregnancy, cocaine use, and the problems encountered by both mother and fetus.  While previous studies have shown that cocaine use during pregnancy impacts the uterus' vasculature, as well as the overall health of mother and fetus, the exact anatomical and physiological impacts have not been determined.  The demand for focus on cocaine use by pregnant women has been steadily increasing since the 1980's.  While the effects of cocaine on the average

person are better understood, the drug's effects on a developing fetus and the uterine structure are less evident. This case may help to define the distribution of cocaine between mother and fetus during heavy cocaine use.

Case History: A 32-year-old gravid 4, para 3 mother in her 36th week gestation was found in the morning sitting on a toilet slumped to the left with her head resting on the sink. A small amount of vaginal bleeding was observed and white powder was found at the scene. Emergency personnel were called and the patient was transported to a regional medical facility. Cardiac monitoring and EKG showed sinus rhythm, but no pulse. No fetal heart tones were detected. Resuscitation efforts were terminated 2 hours later.

Analytical Results:

| | Mother | | Fetus | | |
|---|---|---|---|---|---|
| | Cocaine | BE | Cocaine | BE | |
| Right Heart Blood | 3,756 | 9,368 | 524 | 3,734 | (Heart Blood) |
| Femoral Blood | 1,470 | 11,648 | 1,996 | 18,120 | (Umbilical) |
| Urine | 4,674 | 88,006 | 1,159 | 4,643 | (Kidney) |
| Vitreous | 3,167 | 3,590 | 1,023 | 5,103 | (Liver) |

Analytical data of lung, muscle, brain, adipose, and epidermis (from skin slippage) from the fetus were also obtained.

The mechanism of death is in this case is exsanguination from the ruptured uterus through a previously thinned uterine wall. The approximate 36-week gestation female infant was partially extruded or expelled through the ruptured uterus into the peritoneal cavity. The infant died due to exsanguinating hemorrhage of the mother following rupture of the gravid uterus through the previous C-section scar. Cocaine abuse contributed to the uterine rupture and infant death.

**Uterine Rupture, Cocaine, Exsanguination**

# K28 Collaboration of Emergency Clinician and Forensic Toxicologist in a Suicide Case Related With Amitriptyline

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After attending this presentation, attendees will take into consideration a rapid analysis of amitriptyline from the gastric lavage by High Performance Thin Layer Chromatography (HPTLC) method and confirmed by Gas Chromatography Mass Spectrometer (GC-MS) method during the treatment of a suicide case.

This presentation will impact the forensic science community by highlighting the importance of collaboration between the emergency clinician and forensic toxicologist in a suicide case related with amitriptyline.

A 32-year-old "pharmacist man" was brought to Cerrahpasa Emergency Department by relatives after 3 h ingested 29 tablets of 25 mg Amitriptyline. On presentation, he was noted to have moderate anxiety and semi-cooperative. His vital signs included a temperature of 36.7ᵢC, a blood pressure of 150/100 mm Hg, a pulse rate of 150 bpm, and a respiratory rate of 18 breaths/min. His oxygen saturation was 96% while breathing room air. 12-lead ECG: Sinus tachycardia. His lungs were clear to auscultation bilaterally. His cardiac examination revealed an regular, tachycardic rhythm. There was no discernible murmur. His abdomen was soft, nontender, and nondistended. On his neurologic examination, general depression of all neurologic functions, reduced muscle tone, and tendon reflexes were noted. His Glasgow Coma Score

had fallen to 12/15. After the evaluation, 3000 cc serum physiologic was given via the nasogastric tube in 10 minutes. The analysis of the recovered gastric lavage was done and evaluated in Toxicology Laboratory of Istanbul University, Institute of Forensic Sciences. After gastric lavage, activated charcoal was given and $NaHCO_3$ was applied as antidote treatment. The patient was monitored to observe changes in cardiac rate and conduction and then because of alteration of consciousness and cardiac rhythm, he was admitted to Intensive Care Unit for supportive care.

In the toxicology section, the first washings of gastric lavage was extracted with ethyl acetate:heptane (1:1) in alkaline pH and the analyte was quantified by absorbance/reflectance densitometry using peak-area ratio analysis by HPTLC. Amitriptyline amount was found approximately 26.3 mg/L of gastric lavage sample. Parallel analysis with a GC-MS showed similar quantitative results. This study confirmed the retrospective data of the patient with high doses drug intake according to related articles.

In this case, results obtained by analyzing gastric lavage proved usefulness of the presented method, which represents an enough time to evaluate the epidemiology of the poisonings. Also comparative qualitative analysis between HPTLC and GCMS showed the capability of HPTLC in identifying qualitatively.

**Amitriptyline, HPTLC, GC - MS**

# K29 Fatal Death of an 8-Year-Old Boy From an Explosion Caused by Escaping Butane: Asphyxiation or Death by Explosion?

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After attending this presentation, attendees will be briefed on the sudden death of a small boy involved in an explosion caused by escaping butane.

This presentation will impact the forensic science community by making the attendees aware of pathological and toxicological findings that clarify the cause(s) of death.

Flammable vapors of butane are capable of migrating through an area, creating a path for fire or explosion. Vapors that spread throughout an enclosed space may become flammable or explosive if they encounter an ignition source, whether it be a flame, pilot light, spark, or friction. On the other hand, butane at high concentration can cause asphyxiation as described in several accident or suicide cases.[1]

An 8-year-old boy was found dead under the wreckage of his home which collapsed after an explosion caused by escaping butane. One thousand liters of butane had been contained in a tank in the garden that was found empty. Butane was supplied to the house for domestic use such as cooking and heating. The butane leak inside the home happened during the night and was caused by the accidental turning off of the cooker.

The goal of this forensic investigation was to clarify the cause of death and identify the factors involved in the accident. In fact, considering the large amount of butane which had escaped, it was essential to determine if death was caused by asphyxiation or by

explosion.

Toxicological analyses were performed on blood, liver and fat tissues. Butane was determined in all biological samples by HS/GC-MS. Calibrations were performed in matrix in the case of blood, while the butane amount released by the tissues was estimated using a vial to which a fixed volume of butane gaseous solution had been previously added.

*Autopsy findings:* External examination of the body revealed burn injuries exceeding 80% of the body surface mainly localized on the left part of the face and the thorax. These injuries could not have caused the death of the boy. Internal examination showed extensive head injuries and spinal transection with C2–C3 fractures. Fractures were also revealed in the rib cage, pelvis, and in the arms. Macroscopically, hemorrhagic edemas and passive congestion were evident in lung.

*Toxicological results:* Analyses confirmed that the boy had inhaled butane before death. Butane was revealed in blood at a concentration of 0.78 mg/g, while after headspace extraction, liver and fat tissue released 0.96 mg/g and 0.015 mg/g of butane, respectively. However, these results, mainly those relating to the fat tissue, demonstrate that the butane concentrations, to which the boy had been exposed, were not sufficient to have caused asphyxia.

In fact, aliphatic hydrocarbons, such as butane are lipophilic so that after being taken up from the lungs into blood, they are distributed at high concentrations in lipid-rich tissues such as fat tissues, and also in liver.[1] This characteristic is also confirmed by Kow value of butane (630.96), which, being a measure of hydrophobicity, helps to understand and/or determine the fate of chemicals after exposure. Additionally, these tissue concentrations are generally lower than those reported in butane asphyxiation cases. On the other hand, its determination also in fat tissue demonstrates that the butane leak had been particularly slow because of the body distribution of the toxicant.

Pathological and toxicological findings demonstrated that death occurred after a very short time and was not caused by butane asphyxiation. The victim's injuries localized on the front side indicated that when the accident occurred the boy was standing in front of the source of explosion. Probably, the boy himself had caused gas ignition by switching on the light. However, the autopsy showed that death was not caused by burn injures but was mainly related to the injures caused by the building collapsing.

**Reference:**

1. Sugie H, Sasaki C, Hashimoto C, Takeshita H, Nagai T, Nakamura S, et al. Three cases of sudden death due to butane or propane gas inhalation: analysis of tissues for gas components. Forensic Sci Int 2004 Jul 16;143(2-3):211-4.

**Butane, Explosion, Asphyxia**

## K30 An Unusual Circumstance of Internal Chemical Burn Injury: A Case Report

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After attending this presentation, attendees will understand the injuries due to a rarely reported case of chemical burns due to ingestion of nitric acid in which the history was not of an accidental but of a suicidal nature.

This presentation will impact the forensic community by helping in formulating an emergency treatment protocol.

The present case describes the macroscopic findings of vital changes seen in patient due to ingestion of nitric acid is also highlighted. Spillage of nitric acid (vitriolage) is frequently reported especially in the third-world countries, but an ingestion injury due to nitric acid injuries are seldom encountered in routine practice.

Nitric acid, also known as aqua fortis (strong water) or spirit of nitre or engraver's acid1 is a chemical important for industrial and domestic purposes. A strong acid, powerful oxidizing agent and an ability to nitrate organic material make it an essential in the production of numerous chemicals. Skin contact leads to severe burns and its vapours can cause severe acid burns to the eyes, respiratory tract, and lungs. Being a corrosive, it produces immediate pain and causes burns of mouth, throat, esophagus and abdomen, widespread gastroenteritis, and bloody diarrhoea. Blood may also be found in urine.

A 55-year-old female unable to face the problems of life, ingested an acid around 11:00 a.m. in the morning. She was brought in with complaints of pain and burning sensation and thereby was admitted to a private medical hospital around 3:00 p.m. the same afternoon. She was a known diabetic. Following admission, the patient had undergone laboratory tests which revealed red colored urine (haematuria), proteinuria, aciduria (low urine pH), and pyuria suggesting signs of poisoning and later septic shock.

Amorphous calcium oxalate crystals were also found in urine. Serum electrolytes and other routine investigations were normal. Liver function tests (LFT) showed raised liver enzymes (SGOT = 91, SGPT = 46). Peripheral smear showed a total count of 22,300 (N92 L7 M1) which is a sign of acute inflammation and perforation. This was later confirmed with an abdominal X-ray showing pneumo-peritoneum.

The patient's condition deteriorated after two hours. Arterial blood gas analysis showed acidosis with a pH of 7.1. Serum electrolytes showed variation (serum potas- and whitish tinge of teeth was seen.

Unlike sulphuric acid, when concentrated nitric acid is ingested, the tendency to produce charring of tissues and then perforation is a rare event as recorded in the present case. It may be said that acid burn injuries represent only a minute percentage of burns, but they cause a particular type of lesion in which the morbidity is high and death is certain.

This case presents as an unusual circumstance of an internal burn injury caused due to nitric acid, a rare event, made more so by being used as an agent for suicide. It is recommended that medico-legal death investigators become familiar with the internal chemical burn injuries due to nitric acid.

**Chemical Burn Injury, Nitric Acid, Nitric Acid Ingestion**

## K31 Suicide Cases by Insulin Administration at Tarrant County Medical Examiner's Office

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After attending this presentation, attendees will be briefed on suicide cases by exogenous insulin administration and the measurement of insulin and C-peptide using a new immunoassay in postmortem blood specimen.

This presentation will impact the forensic community by validating this new immunoassay on an automated platform and establishing the normal ranges of insulin, C-peptide, and insulin/C-peptide ratio.

An immunoassay on an automated platform was validated for determination of insulin and C-peptide in postmortem blood specimens. The insulin and C-peptide assays are FDA approved clinical assays by Siemens on the ADVIA Centaur automated platform. This is a common instrument used by many clinical and hospital laboratories. All reagents

were commercially available from Siemens, including calibrators and diluents. Both assays are approved for serum specimens. The manufacturer warns that both assays may have interferences from hemolysis. Postmortem blood specimens typically have gross hemolysis and it is impossible to obtain a clean serum specimen. Thus, postmortem specimen must be pre-treated to reduce the interference enough to obtain reliable values. Dilution of the specimen is the most convenient method that may reduce this interference. Standard addition involves adding a standard in buffer to the specimen, while serial dilution adds assay buffer. Two cases were investigated so far this year. In each case, the sample is a diluted specimen of postmortem whole blood.

The normal range by the automated immunoassay for insulin is 2.6 to 25.0 mU/L and for C-peptide is 0.9 to 4.3 ng/mL. Great than 1 ratio of insulin/ C-peptide is suggestive of exogenous insulin administration.

Case #1: A 52-year-old male with a history of depression and suicide threat was found unresponsive in his parked car. Two empty boxes of humulin insulin were found in the car. The decedent's abdomen reveals numerous injection sites. The postmortem whole blood testing shows exogenous insulin overdose. Subclavian blood insulin is 595 mU/L, C-peptide is 1.66 ng/ml, the ratio of insulin/ C-peptide is 7.56.

Case # 2: A 68-year-old female with a history of diabetes, dementia, depression, and suicidal thought was found unresponsive in her bed. A ¼ empty bottle of insulin was found at home along with a note to her daughter stated, "I love you." The postmortem femoral whole blood testing shows that insulin is 106 mU/L, C-peptide is 1.00 ng/ml, the ratio of insulin/ C-peptide is 2.24.

Values provided by the automated immunoassay platform were compared to values in aliquots of the same samples analyzed by the Mayo Clinical Laboratories (MCL). Twenty samples were tested by both methods. The correlations were 0.992 and 0.996 for insulin and C-peptide, respectively; there was no statistical difference between methods for either analyte. The mean (SD), as mU/L, for insulin was 21.8 (20.6) by MCL and 22.9 (23.5) by automated immunoassay; and for C-peptide, as ng/mL, 2.35 (2.56), and 2.65 (2.99). Thus, the automated platform provided equivalent values to MCL for both analytes.

The recoveries for insulin serial dilution on specimens from both cases ranged from 86% to 115%; the buffer to specimen ratio was 1:4 to 1:16. The recoveries for C-peptide standard addition on specimens from both cases ranged from 85% to 112%; the calibrator to specimen ratio was 1:2. In both cases presented here, the insulin concentrations were too high to use standard addition, while the C-peptide concentrations were too low to use serial dilution.

For the determination of insulin in hemolyzed postmortem specimens, samples can be serially diluted for reliable concentrations. For C-peptide, standard addition can provide reliable concentrations. The dilution was sufficient to reduce the interference caused by the gross hemolysis that occurred postmortem. The above results and case studies indicate that insulin/C-peptide ratio of great than 1 is suggestive of exogenous insulin administration as the cause of death.

**Suicide, Insulin, Insulin/ C-Peptide Ratio**

## K32 Refusing the Refusal: A Review of Texas' Mandatory Blood Draw Initiative

*Chris Heartsill, BS\*, Dallas County Institute of Forensic Sciences, 5230 Medical Center Drive, Dallas, TX 75235*

The goal of this presentation is to reveal the abuse patterns of individuals who refuse to provide a specimen in a DWI investigation. Attendees will understand the levels of drugs and alcohol that are present in the refusal demographic as compared to those who provide a specimen.

This presentation will impact the forensic community by providing toxicologists, investigators, and prosecutors with the impact that the

mandatory blood draw initiative has had across the State of Texas from public awareness to numbers of arrests to prosecution of intoxicated drivers. It will also provide assurance that a mandatory draw program does not improperly target innocent drivers.

It is thought that those who refuse to provide a specimen have experience with driving under the influence of drugs and/or alcohol and their results will be elevated when compared to non-refusals.

The State of Texas is among the leaders in DWI fatalities. By statute, all drivers in Texas have consented to providing a specimen of breath or blood; however, roughly half of all DWI investigations result in the driver refusing to provide a specimen. This has been viewed by the investigating agencies as paramount to hiding evidence in a criminal investigation. Although the Mandatory Blood Draw process has been questioned and challenged by defense attorneys across the State, the process has been upheld in the Court of Appeals in the State of Texas. This has paved the way for a statewide initiative to obtain a search warrant and take a blood specimen when the driver will not voluntarily provide one.

This initiative began with a small agency in North Texas and has spread throughout the State. Most agencies will target certain "peak" times such as holidays or certain weekends to carry out the Mandatory Blood Draw event. During this time media is involved to raise awareness of the event. However, there are agencies that have gone to a full time mandatory system. The process requires the coordination of law enforcement agencies, court administrators, judges, and nurses. Each must be on call throughout the period in which the mandatory event is under way so the warrant can be issued and carried out in a timely manner.

The data suggests that the alcohol concentration is higher on average for those that refuse and the prevalence of drugs is approximately the same. This correlates well with the thought that the refusal drivers have experience with driving under the influence. Statewide alcohol and drug results will be presented comparing historical data from voluntary submissions with these new mandatory specimen results. Arrest statistics, prosecution statistics, and DWI in non-mandatory situations will also be discussed.

**DWI, Mandatory, Blood**

## K33 A Retrospective Study of Drug Prevalence in Alcohol Related Driving Arrests

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The goal of this presentation is to help illustrate some of the difficulties involved in assessing the full impact of drug impaired driving. Officers may not be adequately trained to recognize the symptoms of drug impaired drivers, laboratories may not be performing comprehensive enough testing to identify the drugs that can impair, and prosecutors may not be pursuing the prosecution of drug impaired drivers.

This presentation will impact the forensic science community by providing data to support increased commitment and resources to combat the drug impaired driving problem.

Impaired driving investigations constitute the majority of cases submitted to the NYSP Crime Laboratory's Toxicology section. Both blood and urine are submitted, and the scope of testing is determined by the charges involved and the submitter's request. Alcohol testing is performed by headspace gas chromatography. Drug testing involves an immunoassay screen and a basic drug screen/confirmation by GC-NPD

and GC-MS, plus drug class specific confirmations as warranted. Specimens are retained at least eighteen months after analysis.

A retrospective study was conducted on blood specimens that had exceeded the eighteen month retention time. Over 300 samples from 2005 and 2006 were tested. The samples were screened by ELISA for the following eight drugs/drug classes at the indicated cut-offs: cannabinoids (10 ng/mL), cocaine/benzoylecgonine (50 ng/mL), opiates (20 ng/mL), benzodiazepines (50 ng/mL), methamphetamine/MDMA (20 ng/mL), methadone (50 ng/mL), carisoprodol (500 ng/mL), and zolpidem (25 ng/mL).

The study involved cases that had only alcohol testing performed originally. The first set of samples involved charges/requests related only to alcohol. The subsequent ELISA drug testing conducted in this study revealed 39% were presumptive positive for one or more drug/drug class. The majority, 30% of the total cases, were presumptive positive for cannabinoids. The second set of cases involved charges/requests for alcohol and drugs, but only alcohol testing was performed. Laboratory policy dictates that alcohol testing be conducted first. If the result is ≥0.11% by weight, results are reported with a statement indicating that if drug testing is still needed, you must contact the laboratory. The additional testing was not requested for these cases. However, when ELISA drug screening was performed for this study, 48% of these cases were presumptive positive for one or more drug/drug class, primarily cannabinoids.

An additional part of this study involved cases with charges/requests related to drugs, where drug testing was originally performed (some may have also included alcohol testing with results < 0.11% by weight). Since the original testing of these cases, the protocol for immunoassay drug screening in the laboratory changed from FPIA to ELISA, more assays were included, and many cutoffs were lowered. These cases were included in the study to assess the significance of these changes.

**Drugs, Driving, Cannabinoids**

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## K34     PCP and Drug Impaired Driving in San Francisco, California

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After attending this presentation, attendees will become aware of the effects that phencyclidine has on driving skills as demonstrated by case examples from drivers arrested for DUID by PCP in a three year period.

This presentation will impact the forensic science community by offering a set of reference data on PCP concentrations often measured in impaired drivers and epidemiological data of signs and symptoms associated with PCP intoxication and impairment.

In this study, demographic profiles, drug concentrations of PCP, and typical observed behaviors of subjects arrested for driving under the influence are presented where PCP was a significant toxicological finding from cases submitted to the Toxicology Laboratory of the Forensic Laboratory Division of the Office of the Chief Medical Examiner, City and County of San Francisco. Phencyclidine, PCP, was first developed in 1956 by Park Davis and investigated as a possible anesthetic. In clinical trials, some patients experienced a prolonged post-operative psychosis and it was withdrawn from clinical use in 1965. It is this adverse affect and the dissociative hallucinogenic properties of PCP which contributed to its popularity as a drug of abuse in the late

1960s. PCP use steadily declined over the next few decades, but recent data suggests there is resurgence in its use. The Drug Abuse Warning Network (DAWN) has presented data indicating that since 1999, there has been a general increase in PCP related visits to Emergency Departments.

All blood samples collected from impaired drivers are screened for ethanol and in those cases where ethanol concentrations are below 0.08 and drugs are suspected, a drugs abuse panel may be requested by the submitting agency. ELISA screening (Venture Labs, Inc.) was performed for amphetamine, barbiturates, benzodiazepines, cocaine, fentanyl, methadone, methamphetamine, opiates, oxycodone, phencyclidine, propoxyphene, and tricyclic antidepressants. Screened positives are confirmed by GC-MS.

In 2005, the SF-OCME's toxicology laboratory investigated 209 cases of suspected driving under the influence of drugs and 3 were positive for PCP (an incidence of 1.4%). In 2006 there were again 3 positive PCP drivers out of 183 submitted cases (an incidence of 1.6%) and in 2007 there were 6 PCP positive cases out of 170 submitted (an incidence of 3.5%). Reported here is the data from 13 PCP positive drivers, who were arrested for drug impaired driving. They were predominantly male (92%), had a mean and median age of 40, and in 62% PCP was the only psychoactive drug detected. The mean PCP concentration was 0.09 mg/L (range (0.03 – 0.20 mg/L). PCP positive drivers were significantly impaired with marked sedation, slurred speech, and when performed, subjects did poorly on field sobriety tests.

The incidence of driving under the influence of PCP in San Francisco is low but appears to be increasing (1.4% in 2005 to 3.5% in 2007), at the same time that the number of drivers for whom blood was submitted to the laboratory declined. The average age of PCP drivers is higher than the average age of other drug impaired drivers and higher than PCP impaired drivers reported from other jurisdictions. The drug concentrations appear to be higher in San Francisco than those reported in other studies. In conclusion, PCP continues to be found in San Francisco drivers arrested for DUID, thus making it important to continue screening suspected drug impaired drivers for PCP as they tend to be both severely impaired and have little or no ability to safely operate a motor vehicle.

**PCP, Impaired Driving, San Francisco**

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## K35     Sensitive Method For Detection of Cocaine and Metabolites by Liquid Chromatography Tandem Mass Spectroscopy (LC - MS/MS) in Urine

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After attending this presentation, attendees will be able to understand the uses of an assay for cocaine and metabolites with a lower limit of detection.

This presentation will impact the forensic science community by describing a method with a low limit of detection for cocaine and metabolites that will improve the ability to investigate drug use patterns.

Cocaine (COC) is an alkaloid found in *Erythroxylon coca*, and is a potent CNS stimulant that results in a state of increased alertness and euphoria. Cocaine can block the reuptake of neurotransmitters norepinephrine, dopamine, and serotonin. COC is rapidly metabolized to major metabolites like benzoylecgonine (BE) which is further metabolized to minor metabolites like m-hydroxybenzoyleconine (HOBE) and Norcocaine (NC). Cocaethylene (CE) is formed by trans-

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esterification of cocaine with ethyl alcohol when used simultaneously. Anhydroecgonine methyl ester (AEME) is a unique metabolite of smoked cocaine, and anhydroecgonine ethyl ester (AEEE) has been identified in cocaine smokers who also use ethyl alcohol. A method has been developed for the detection of and quantiation of COC, HOBE, AEME, AEEE, NC, CE, and BE in urine at low concentrations.

Sixty-eight randomly selected previously confirmed BE positive by GC-Ms were collected from May 19th to 22nd and June 20th to 27th. Samples were extracted using SPE and 30uL of the reconstituted extract was injected. Chromatographic separation was achieved using a gradient consisting of Mobile phase A [20mM ammonium formate (pH = 2.7)] and Mobile phase B [50%/50% methanol/acetonitrile], and an XDB-C8, 1.8micron, 2.1x50mm column, with a flow rate of 270 ul/min. Concentrations were calculated by comparing the drug peak area with the internal standard peak-area. The ratio was plotted against a standard curve.

The assay displayed linearity from 1.0m- 1000 ng/mL for all analytes. Between-run CVs were <10% throughout the linear range. Of the 68 BE positive samples, 56 were positive for cocaine, 32 for AEME, 7 for AEEE, 64 for HOBE, 29 for NC, and 32 for CE at > 1.0 ng/mL.

Standard surveys to determine methods of cocaine use rely on individual responses to survey questions, and assumes that users responses are honest. We report here on a sensitive method to identify cocaine metabolites at concentrations as low as 1.0 ng/mL. Because of the unique derivation of some of the metabolites, the pattern of the metabolites makes it possible to determine the user's method of cocaine ingestion. This assay could be used to validate or challenge current survey techniques.

**Cocaine, Cocaine Metabolites, LC-MS/MS**

## K36  Opiate Withdrawal and Adulterated CNS Depressant Drug Impaired Driving: Case Interpretation and Overcoming Motions to Suppress

*Michael Wagner, PhD\*, State Department of Toxicology, Indiana University, 550 West Sixteenth Street, Indianapolis, IN 46202*

After attending this presentation, attendees will understand the key medical legal issues addressed when evaluating impaired driving resulting from opiate addiction. Case history, self reporting, biological samples, analytical test results, relevant case law, and strategies challenging motions to suppress will be discussed. In addition, the opposing conclusions from the expert witnesses will be presented.

This presentation will impact the forensic community by interpreting DUID cases involving impairment when only trace analytical test results are available.

On March 7th at 1445 hours, police are notified of a collision involving two vehicles (vehicle #1 a 1998 Nissan Frontier (pickup truck) and vehicle #2 a 1987 Volvo 740). The weather was clear and dry. Vehicle #2 was traveling eastbound and made a left hand turn (northerly) when it was struck broadside by vehicle #1 that was traveling westbound. Vehicle #1 driven by a 47-year-old male had violated a red traffic control device just prior to this collision. As a result of this accident, two males ages 17 and 34 occupying vehicle #2 had been fatally injured.

Scene evaluation of the driver for vehicle #1 reveals the subject claims to have been reaching down for a water bottle and taking his attention off the roadway. The subject made statements at the scene to police officers and medical personnel that he was a heroin addict and had ingested three Klonopin® prescription pills approximately 09:00 – 09:30. He crushed two of the pills and snorted them, and then he ingested the third. Subject claimed to have eaten very little in the past

two days (two bowls of cereal the previous day) and nothing that day (body height and weight were 6'1" and 135 pounds). In addition, the subject claimed to be in withdrawal with his last use of Heroin having been on March 5th. The subject admitted to be in route to purchasing more Heroin and having $2,500 dollars in cash. Multiple blood samples were collected and indicated the presence of trace parent cocaine and metabolite, trace clonazepam, while urine test results confirmed the presence of morphine and oxycodone in addition to parent cocaine and benzoylecognine.

**DUID, Opiate Impairment, Benzodiazepines**

## K37  Prescription Drugs, Poor Driving, DRE Evaluation…and a Surprising Verdict – A DUID Case Study

*Laura J. Liddicoat, BS\*, Toxicology Section - WSLH, 2601 Agriculture Drive, PO Box 7996, Madison, WI 53707-7996*

After attending this presentation, attendees will have a greater understanding of drug interpretation, prosecutor preparation, and effective expert witness testimony for prescription drug impaired driving cases.

This presentation will impact the forensic community by influencing toxicologists who are involved with suspected DUID cases by enhancing their understanding of the challenges to interpretive issues.

For drugs other than alcohol, interpretation of drug concentrations and effects on safe driving ability is extremely complex. The toxicologist must consider drug pharmacology, pharmacokinetics, drug interactions, medical information, and research findings and apply them to the individual case scenario. This information must then be presented to the attorneys during preparation for the trial.

The case study that will be presented involves several drugs that can severely affect driving abilities. The drugs include oxycodone (at a potentially toxic concentration of 530 ng/mL), diazepam, nordiazepam, cyclobenzaprine and citalopram. Poor driving was observed by a citizen driver, reported to law enforcement, and documented by the arresting State Patrol Officer. A Drug Recognition Expert (DRE) was called to the scene and conducted an evaluation of the driver. The DRE concluded that he was impaired and under the influence of a CNS Depressant and Narcotic Analgesics.

At trial the driver alleged that he was able to ingest several oxycodone pills while in custody and prior to the blood sample collection. Even though this case had all the required elements for a DUID conviction, the first hearing resulted in a mistrial and was subsequently retried. The full case will be presented with emphasis given to drug interpretation, pharmacokinetics, prosecutor preparation, and effective expert witness testimony.

**Drugs, Driving, Impairment**

## K38  Driving Under the Influence of Methamphetamine in the City & County of San Francisco, California

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After attending this presentation, attendees will have a better understanding of the signs and symptoms often observed in drivers driving under the influence of methamphetamine, the measured concentrations of the drug in the drivers' blood specimens, and the

incidence of methamphetamine in alleged DUID drivers in San Francisco, California.

This presentation will significantly impact the forensic community by providing reference epidemiological and toxicological data drawn from actual driving cases which can serve as tools in the investigation of alleged DUID cases involving methamphetamine in the United States and abroad.

In San Francisco, California, suspected DUID drivers are charged with violating Vehicle Code Section 23152(a) which relates to driving while a person's physical or mental faculties are impaired by alcohol (or drugs) to the extent that they are "unable to drive their car with the same caution characteristic of a sober person, of ordinary prudence, under the same or similar circumstances." A separate charge, 23152(b), relates to driving with BAC equal or greater to 0.08% (w/v). In this study we present the drivers' demographic profiles are presented together with the concentrations of methamphetamine, amphetamine, and related compounds in biological specimens submitted to the toxicology laboratory of the Forensic Laboratory Division of the SF OCME.

A computerized database (NIKTOX) and a manual search of reports were used to identify DUID cases in which methamphetamine and/or related compounds were detected and confirmed/quantified in biological specimens during a 3-year period (2005-2007).

In 2005, there were 209 cases of drivers suspected of driving in violation of 23152(a). Their age ranged from 17 to 82 years (median: 33 years). 171 of these drivers were male (82%). Methamphetamine was found in 17 cases and the age of those drivers ranged from 20 to 63 years (median: 37 years). 88% of the methamphetamine positive cases involved male drivers (n=15). Blood was collected in only 3 of the 17 cases. In the three blood cases, the methamphetamine and amphetamine concentrations were 0.6, 1.5, and 0.3 mg/L and 0.1, <0.1, and <0.1 mg/L, respectively.

In 2006, there were 183 cases of drivers suspected of driving in violation of 23152(a). This represented a decrease of 12% from the previous year. Their age ranged from 19 to 73 years (median: 33 years). Of these drivers, 157 were male (86%). Methamphetamine was found in 21 of the 183 cases. This represented an increase of 3.4% in methamphetamine incidence as compared to the previous year. The age of these 21 drivers ranged from 19 to 51 years (median: 28 years). Male drivers represented 71% of the methamphetamine positive cases (n=15) and 29% involved female drivers (n=6). The percentage of female drivers involved in methamphetamine DUID cases in 2006 represented more than a two-fold increase from the previous year. Blood was collected in 10 of the 21 cases and the median methamphetamine and amphetamine concentrations measured were 0.4 mg/L (range: <0.1 to 0.8 mg/L) and 0.1 mg/L (<0.1 to 0.1 mg/L), respectively.

In 2007, there were 170 cases of drivers suspected of driving in violation of 23152(a). This represented a further decrease of 7% in submissions as compared to the previous year. The drivers' age ranged from 17 to 82 years (median: 33 years). Of these drivers, 135 were male (79%). Twenty-five of the 170 cases were found to contain methamphetamine, a further increase of 3.2% in methamphetamine incidence from the previous year. The age of these 25 drivers ranged from 19 to 51 years (median: 33 years). Of these cases, 84% involved male drivers (n=21). Blood was collected in 12 of the 25 cases and the median methamphetamine and amphetamine concentrations measured were 0.3 mg/L (range: <0.05 to 0.7 mg/L) and 0.1 mg/L (range: <0.05 to 0.1 mg/L), respectively.

Methamphetamine incidence in driving under the influence cases almost doubled between 2005 and 2007 (from 8.1% to 14.7%) but in the same period the total number of DUID laboratory submissions by law enforcement agencies decreased by almost 19%. This suggests that driving under the influence of methamphetamine in San Francisco is on the rise but law enforcement agents in this jurisdiction may not be adequately resourced or adequately trained in the recognition and interception of drivers driving under the influence of substances other than ethanol. Additionally, women and younger drivers appear to be increasingly involved in methamphetamine DUID cases. It may be that greater efforts should be made in further educating our population of the risks associated with methamphetamine use and abuse instead of exclusively relying on the deterrent effects of fines and other penalties.

**Methamphetamine, Driving, San Francisco**

## K39 Use of Serotonin Metabolites in Postmortem Alcohol Determinations

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After attending this presentation, attendees will more fully understand postmortem ethanol formation and ways to identify it.

Specimens from aviation accident victims are submitted to the FAA's Civil Aerospace Medical Institute (CAMI) for toxicological analysis. During toxicological evaluations, ethanol analysis is performed on each such case. Care must be taken when interpreting a positive ethanol result due to the potential for postmortem ethanol formation. Historically, ethanol distribution in various tissues and fluids from the same case and/or the presence of other volatile organic compounds at abnormal concentrations in these fluids and tissues has been employed as an indicator of postmortem microbial ethanol formation. However, these methods are not always reliable. The consumption of ethanol has been shown to alter the concentration of two major serotonin metabolites, 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA). While the 5-HTOL/5-HIAA ratio is normally low, previous studies have demonstrated that the urinary 5-HTOL/5-HIAA ratio is significantly elevated following ethanol ingestion. The 5-HTOL/5-HIAA ratio is not affected by the microbial formation of ethanol, by consumption of serotonin-rich foods or by the use of SSRI's.

A single analytical approach has been developed to determine concentrations of both 5-HTOL and 5-HIAA that has provided a convenient, rapid and reliable solution to this problem. This novel methodology eliminates the need for two separate and unrelated analytical techniques, GC/MS and LC/EC, for the determination of these metabolites. The simultaneous determination of 5-HTOL and 5-HIAA in forensic urine specimens was achieved using a liquid/liquid extraction technique in conjunction with LC/MS. The ion trap MS used allowed us to perform MS/MS/MS on both 5-HTOL and 5-HIAA, and afforded limits of quantitation below 1 ng/mL for each compound. After development of this method, the previously established, antemortem, 15 pmol/nmol 5-HTOL/5-HIAA ratio cutoff was investigated and subsequently validated for use with forensic specimens.

The FAA laboratory utilizes this method to examine all postmortem ethanol-positive urines, where the source of ethanol is unclear. This presentation will discuss the difficulties in determining the source of ethanol in postmortem cases, markers of ethanol ingestion, and the application of this novel methodology in elucidating ethanol origin. Multiple case studies that involved postmortem alcohol formation will be presented.

**Postmortem Ethanol, LC/MS, Serotonin Metabolites**

## K40 Clinical and Forensic Toxicology of

## Gamma - Hydroxybutyrate Closely Resembles That of Ethanol

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After attending this presentation, attendees learn about the clinical and forensic toxicology of two widely used recreational drugs, namely the legal drug ethanol and the illicit drug gamma-hydroxybutyrate (GHB). Both substances are highly soluble in water have low molecular weight and their pharmacological effects are similar to the major central nervous system depressants (general anesthetic gases, barbiturates and benzodiazepines).

This presentation will impact the forensic community by teaching the similarities and differences in clinical pharmacokinetics of ethanol and GHB, the analysis and stability of these substances in blood during storage, the distribution between serum and whole blood, and the effects of food and gender on concentration-time profiles. Moreover, the toxicity of ethanol and GHB are compared and contrasted based on the concentrations determined in blood from impaired drivers and medical examiner cases.

Ethanol and GHB are produced naturally in the body and are measurable in blood and urine at very low concentrations of ~1 mg/L. For recreational purposes both drugs are taken orally and are rapidly absorbed from the gut and distributed into the total body water (TBW) compartment. The distribution of ethanol and GHB between the plasma and erythrocyte fractions of whole blood is similar to that of water distribution, suggesting serum/whole blood ratios of 1.15:1 (range 1.10 to 1.20). Ethanol and GHB don't bind to plasma proteins and undergo extensive hepatic metabolism with only a small fraction (2-5%) of the dose being recoverable in the urine. The metabolism of ethanol and GHB occur by capacity limited kinetics and mathematically this can best be described by the Michaelis-Menten equation. Human dosing studies have shown that when the concentrations in blood pass 150 mg/L (ethanol) and 10 mg/L (GHB), the metabolizing enzymes are virtually saturated with substrate and zero-order kinetics applies. After moderate doses, the elimination rate of ethanol from blood is within the range 100-200 mg/L/h compared with 10-20 mg/L/h for GHB. The terminal half-lives of ethanol and GHB are relatively short; being in the range 15-30 min. The apparent volumes of distribution ($V_d$) of both substances are 0.5-0.7 L/kg as expected for water-soluble, non-protein bound drugs that distribute into the TBW. Concentration-time profiles of ethanol and GHB after moderate doses were similar for men and women in terms of $C_{max}$, $t_{max}$ and area under the curve (AUC). The rate and extent of absorption is slowed considerably if ethanol or GHB are ingested together with or after a meal, owing to delayed gastric emptying and first-pass metabolism. Under these conditions, $C_{max}$, $t_{max}$ and AUC are markedly diminished compared with the same dose of the drugs taken on an empty stomach.

Both ethanol and GHB can be determined in blood and urine by conventional gas-liquid chromatography with a flame ionization detector, either by direct injection or headspace technique. Methods are also available for analysis of these substances by GC-MS, which permits use of deuterium labeled analogues as internal standards for unequivocal identification. The concentrations of ethanol and GHB in specimens of whole blood from impaired drivers were remarkably stable during storage at 4ºC for several months after sampling.

The mean and median blood-ethanol concentrations in impaired drivers were 1,700 mg/L (N = 29,000) and in some instances the concentrations exceeded 4000 mg/L. These results can be compared with mean and median GHB concentrations of 89 and 82 mg/L (N = 548) in impaired drivers, highest 340 mg/L. The concentrations of ethanol and GHB in blood from living subjects overlapped with concentrations seen in drug-related deaths. The mean and median blood-ethanol concentration (N = 800) was 3600 mg/L and 3500 mg/L, respectively compared with mean and median GHB (N = 37) of 294 mg/L and 190 mg/L, respectively.

Capacity limited pharmacokinetics of ethanol and GHB needs to be carefully considered when the concentrations in blood after toxic doses are interpreted. The terminal half-life should not be used to make predictions about times necessary to eliminate ethanol or GHB from blood or the amount ingested after large recreational or abuse doses are taken. Interpreting the concentration of ethanol and GHB in medical examiner in terms of toxicity and whether drug intoxication was a possible cause of death is complicated by concomitant use of other psychoactive substances.

**Ethanol, GHB, Toxicology**

## K41 Determining Concentrations of Fentanyl in Decomposing and (Formalin-Stored) Postmortem Liver Tissue Over Time by Gas Chromatography-Mass Spectrometry (GC-MS)

*Tiffany L. Eckert-Lumsdon, MS\*, Johnson County Sheriffs Crime Lab, 6000 Lamar, Mission, KS 66202; and Jim Mcgill, PhD, Southeast Missouri State University, One University Plaza, Cape Girardeau, MO 63701*

After attending this presentation, attendees will have an overview of the opioid analgesic drug fentanyl, its stability over time in aqueous and liver matrices, and the effects of simulated embalming in formalin on its concentration.

This presentation will impact the forensic community by aiding the forensic medical examiner and forensic scientist in the determination of fentanyl concentrations in decomposing tissue cases as well as cases where tissue has been stored in formalin.

A systematic study of matrix effects on the postmortem concentration of the opioid analgesic drug fentanyl in liver tissue was conducted over a six-year period. Porcine liver homogenates were spiked with 200 nanograms of fentanyl per gram of liver to simulate a fatal overdose and treated with the chemical preservative formalin to simulate embalming of the deceased victim. The samples were prepared in triplicate (samples 1A-1C) and stored at room temperature. Periodically, aliquots were removed from the sample containers and extracted using a solid-phase extraction (SPE) method, and the concentration of fentanyl was monitored over time by gas chromatography-mass spectrometry (GC-MS). To isolate the effects of formalin and of the liver tissue itself on fentanyl's concentration, triplicate samples were also prepared in which these two components were systematically omitted from the sample sets (samples 2A-4C). Also, negative controls were prepared in which no fentanyl was spiked into the samples (samples 5A-6C). Statistical analysis of the concentration data over time was conducted to determine effects of time and other sample matrix components on fentanyl concentrations. Details of the study, data analysis, and results as well as implications for forensic toxicology practice will be presented.

**Fentanyl, GC-MS, Solid-Phase Extraction, Formalin**

## K42    Homicide by Propofol

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After attending this presentation, attendees will understand how medications such as propofol can be used to murder individuals, and the investigative techniques available to identify such deaths.

This presentation will impact the forensic science community by describing a unique and difficult to investigate method of homicide.

In November 2005, a 24-year-old previously healthy woman was found dead in her residence in Gainesville, Florida after her boyfriend had been unable to reach her. She was found prone, facedown in the bed, and was fully clothed. No drugs or drug paraphernalia were found in the residence, and there were no signs of a struggle or other interpersonal violence. At autopsy, the body had fixed lividity on the ventral surfaces, with blanched areas on the forehead, nose, chin, and across the chest corresponding to the left arm under the body. A single, minute pinpoint puncture wound was identified on the left antecubital fossa, directly overlying a prominent subcutaneous vein in the antecubital fossa. Minimal hemorrhage was present in the intervening soft tissues. No other abnormalities were observed during the autopsy. Blood, urine, vitreous humor and tissue specimens were obtained and submitted for toxicological and histological studies.

Law enforcement personnel in attendance at the autopsy alerted those at the scene regarding the puncture wound, and subsequently, the investigation widened to include inspection of garbage containers outside of the residence. Investigators found vials of propofol, etomidate, midazolam, and saline, along with needles and intravenous prep materials.

The medications and medical paraphernalia were traced back to a local hospital and linked to a male acquaintance of the victim. The male acquaintance, who apparently was infatuated with the young woman, was an ICU nurse who coincidentally was terminated from his position shortly after the young woman's body was found. Although he was suspected to have involvement in this victim's death, several months passed before DNA evidence definitively linked him to the crime. During this intervening time, he left the region, and subsequently fled the country for Ireland.

Postmortem blood and urine specimens were subjected to comprehensive drug analysis including volatiles and over-the-counter, prescription and illicit drugs. The blood was positive for propofol (4.3 mg/L), phentermine (0.64 mg/L), and diphenhydramine (trace). In addition, 15 mg/L of GHB was detected in the urine.

Propofol is an intravenous anesthetic agent with rapid-onset of action and is primarily used for the induction and maintenance of anesthesia in surgical procedures, as well as a sedative in various clinical settings. Blood concentrations of propofol at approximately 4 mg/L are typically achieved for maintenance during major surgery, and individuals at these concentrations require mechanical ventilation.

With these results, in addition to the absence of significant anatomic findings, the death was certified as propofol intoxication, and the manner of death was certified as homicide. Pursuant to the conclusion of the medicolegal death investigation, a warrant was issued for the male acquaintance's arrest. Eventually he was captured in Senegal, extradited to the U.S., and formally charged and tried for the death of the young woman. The male acquaintance was found guilty of first-degree murder and sentenced to life without parole.

**Homicide, Propofol, Death Investigation**

## K43  Five Fatal Occupational Injuries Due to Gas Inhalation Occurred During Truck-Tank Washing Operation:  Environmental Findings

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After attending this presentation, attendees will be briefed on five cases of fatal asphyxiation at work, which occurred during a truck-tank washing operation.

This presentation will impact the forensic community and/or humanity by demonstrating how fatal deaths were caused by gaseous hydrogen sulfide ($H_2S$), a byproduct of the chemical compound being shipped, and that the accumulation of toxic gases in a closed space can induce asphyxiation in a matter of minutes.

During a routine truck-tank washing operation, a worker got into the empty tank through the upper pothole and in a few minutes fell unconscious. Assuming an accident happened, a second worker went into the tank where he also fell unconscious. The last three men died trying to remove their co-workers out of the tank. Before the washing operation, the tank had previously contained sulfur liquid. All five workers had been in good health and had had a mean age of 37.6 years (range 20-64).

To clarify the cause of death and identify the factors involved in asphyxia, it is crucial to identify the fatal compound(s) and its/their origin. Therefore, several specimens from the tank were characterized and a simulation on two analogous truck-tanks was also carried out.

Dregs of a blackish liquid and a yellowish granular solid from the tank bottom were analyzed using headspace/GC-MS technique. Air samples were analyzed using commercial available color dosimeter tubes and $H_2S$ quantitative determination was also performed in liquid sulfur. Thiosulfate, was measured in blood samples by GC/MS technique after derivatization with pentafluorobenzyl bromide.

Analyses confirmed that the dregs of yellowish solid samples were composed of sulfide. The blackish liquid was a mixture mainly consisting of liquid sulfide and $H_2S$ as contaminant (2.5 mg/l). The absence of hydrocarbon-aliphatic compounds and solvents together with its almost neutral pH (7.6) demonstrated that the workers had not used detergents or basic compounds.

Air monitoring at the third opening inside the tank (one week after the accident), revealed high $H_2S$ concentration (> 60 ppm) while sulfur oxides were negligible, which excluded a sulfur combustion induced by the workers. At the fourth opening (one month after the accident), $H_2S$ air concentration was less than 0.25 ppm. This depletion was due to the continuous opening of the porthole during the rescue operation and the following inspections, as demonstrated by the strong characteristic odor of rotten egg that could be smelled in the area outside the truck-tank. The high $H_2S$ concentration in the air inside the tank was ascribed to the contamination of the original liquid sulfur, produced by Claus's process, the most significant industrial process used to recover elemental sulfur from gaseous hydrogen sulfide.

To support the hypothesis that $H_2S$ rising from liquid sulfur was responsible for the deaths, two similar truck-tanks used for liquid sulfur transport, and the sulfur itself were also tested. Before loading liquid sulfur, air inside the tanks contained only $O_2$ (20.9 % v/v). During the

loading phase H$_2$S air concentrations were 41 and 71 ppm, respectively and became 80.9 and 600 ppm when the tanks were fully loaded.

The liquid sulfur analyzed revealed high contaminations of H$_2$S: 85 and 108 mg/Kg.

According to Henry's Law (K$_H$=0.087 Pa•m$^3$/mol), H$_2$S tends to pass towards the gas phase. This evaporation is favored by the movement of the liquid (e.g. during the shipping) that increases the kinetics of evaporation. Therefore, after liquid sulfur had been removed from the tank bottom, H$_2$S remained in the gas phase causing the asphyxiation of the workers, as confirmed by the pathological and toxicological findings. In fact, abnormal concentrations of thiosulfate, the major metabolite of H$_2$S, from 0.023 to 1.63 mmol/l (average value: 0.38 mmol/l) were revealed in all *postmortem* blood samples.

Environmental and biological results confirmed that H$_2$S fumes were responsible for the multiple deaths and no other adverse reactions that happened inside the tank. This report presents valuable findings in correctly identifying the cause of death in gas asphyxiation cases.

**Asphyxia, Hydrogen Sulfide, Occupational Accident**

# K44 Nine Xylazine Related Deaths in Puerto Rico

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After attending this presentation, attendees will understand the nature of lethal risks posed by xylazine and will be familiarized with toxicological and pathological findings of nine xylazine related deaths. The presentation will raise awareness among the forensic community, law enforcement, clinicians and the general public regarding the potential lethality of xylazine used alone or in combination with other drugs.

This presentation will impact the forensic community by providing a better understanding regarding the lethal risks of xylazine and increasing awareness of the presence of this substance as an adulterant of illicit drugs and its role as a cause of death.

Xylazine is a phenilaminothiazine derivative, structurally related to clonidine that is employed as a veterinary sedative, analgesic, and anesthetic that has been proven harmful to humans. In the mid 1960's xylazine was investigated as a sedative hypnotic/analgesic premedication in humans, but was rejected because of its frequent association with severe hypotension. In humans, toxicity consists of central nervous system depression, bradicardia, and hypotension. Its pharmaceutical action results in sympathetic discharge via stimulation of alpha-2-adrenoreceptors.

Xylazine has been frequently found as an adulterant of illicit drugs, mainly heroin. Both drugs are dangerous to humans, and due to their similar pharmacologic effects, drug synergy can occur.

Researchers reviewed nine cases occurring within the period 2003-2007 at the Puerto Rico Institute of Forensic Sciences (PRIFS) in which xylazine was detected and determined to be the cause of death. Xylazine was detected and quantified in blood using Liquid Chromatography/Mass Spectrometry (LC/MS).

The nine cases of xylazine related deaths are summarized in Table 1. In eight of the nine cases, the individuals were found unresponsive and pronounced dead at the scene. The scene was the decedent's residence in five of the eight cases, in two cases it was the street, and in one case a hospital room. Case #9 complained of shortness of breath, had a witnessed collapse at his residence, and died minutes later. History

of drug abuse was present in all cases. Five were males and four were females whose ages ranged from 23 to 70 years. At autopsy there was no external or internal trauma in any of the cases. Recent venipuncture sites in the upper extremities were found in two cases. Internal examination was remarkable for moderate to severe pulmonary congestion and edema, a common finding for all cases. No additional pertinent autopsy findings were noted. Toxicological analyses disclosed the presence of blood xylazine levels (range 0.29 – 5 μg/mL) and morphine (range 0.08 – >1 μg/mL) in all cases. Cocaine was detected in three cases and ethanol in four cases. The cause of death was determined to be toxic effects of xylazine and opioids for all cases. Additionally cocaine and alcohol were included in cases in which they were detected. The manner of death was accidental in all nine cases.

According to a recent study of the street heroin samples analyzed by the Control Substance Laboratory of the PRIFS, xylazine was found in 36% of the cases as a heroin adulterant. Given the potential toxicity and lethality of xylazine when used alone or in combination with heroin or other drugs, it is necessary to be aware of the emergence of this substance in the community and consider methods of improving its detection. There are limited reports of human toxicity and deaths related to xylazine. The toxicological and pathological aspects of nine cases are reported and discussed with all of the literature available to date.

**Table 1: Nine PRIFS Xylazine Related Deaths**

| Cases | Gender | Age | Drugs | Blood (μg/mL) |
|---|---|---|---|---|
| 1 | M | 29 | Xylazine | 0.29 |
| | | | Morphine | 0.17 |
| | | | Cocaine | 0.13 |
| | | | Benzoylecgonine | 0.66 |
| 2 | F | 35 | Xylazine | 0.50 |
| | | | Morphine | 0.21 |
| | | | Benzoylecgonine | 0.13 |
| | | | Ethanol | 0.20% |
| 3 | M | 23 | Xylazine | 0.70 |
| | | | Morphine | 0.07 |
| 4 | F | 45 | Xylazine | 0.70 |
| | | | Morphine | >1.00 |
| | | | 6-Monoacetylmorphine | 0.03 |
| | | | Codeine | 0.08 |
| 5 | M | 53 | Xylazine | 0.30 |
| | | | Morphine | 0.26 |
| | | | 6-Monoacetylmorphine | 0.01 |
| | | | Cocaine | 0.10 |
| | | | Benzoylecgonine | >1.00 |
| | | | Ethanol | 0.14% |
| 6 | F | 70 | Xylazine | 1.90 |
| | | | Morphine | 0.08 |
| | | | Cocaine | 0.19 |
| | | | Benzoylecgonine | 0.43 |
| 7 | M | 49 | Xylazine | 5.00 |
| | | | Morphine | 0.27 |
| | | | 6-Monoacetylmorphine | 0.01 |
| | | | Ethanol | 0.12% |
| 8 | F | 30 | Xylazine | 2.00 |
| | | | Morphine | 0.43 |
| | | | 6-monoacetylmorphine | 0.02 |
| | | | Benzoylecgonine | 0.11 |
| | | | Ethanol | 0.36% |
| 9 | M | 30 | Xylazine | 0.41 |
| | | | Morphine | 0.13 |
| | | | 6-Monoacetylmorphine | 0.02 |
| | | | Benzoylecgonine | 0.10 |

**Xylazine, Heroin, Cause of Death**

## K45    Common Heroin Adulterants in Puerto Rico: The Emergence of Xylazine

*Kazandra Ruiz, BS\*, and Luz A. Silva Torres, BS, Puerto Rico Institute of Forensic Sciences, PO Box 11878, San Juan, Puerto Rico 00922-1878; and Carlos F. Chavez-Arias, MD, Puerto Rico Institute of Forensic Sciences, PO BOX 11878, Caparra Heights Station, San Juan, Puerto Rico 00922-1878; Osca Peralta Narvaez, BS, Puerto Rico, Institute of Forensic Sciences, PO Box 11878, San Juan, Puerto Rico 00922-1878; Flor Mattos De Jesus, BS, Institute of Foresic Science, Calle Maga Esq. Casia, #9 Urb. Reparto Metropolitano, San Juan, Puerto Rico 00922; and Joseph Bloom-Oquendo and Jose Rodriguez-Orengo, PhD, Puerto Rico Institute of Forensic Sciences, PO BOX 11878, San Juan, Puerto Rico 00922-1878*

After attending this presentation, attendees will learn about common adulterants of heroin and the emergence of xylazine as the main adulterant of heroin in Puerto Rico. The goal of this study is to increase the awareness about the appearance of these drug combinations and their potential toxic effects.

This presentation will impact the forensic community by presenting statistical information about the use of xylazine, a drug that was identified as the most frequent adulterant of the street heroin in Puerto Rico.

Xylazine is marketed as a veterinary drug and used as a sedative, analgesic, and muscle relaxant for large animals, such as deer, ruminants, and horses. Xylazine is not approved for human use because it has been proven harmful to humans. Only 27 cases of toxicity caused by xylazine consumption have been documented in humans. According to these reported cases, consumption was accidental, suicidal or for homicidal purposes, occasionally resulting in death. Xylazine was detected and reported as the cause of death in nine postmortem cases from the Puerto Rico Institute of Forensic Sciences (PRIFS).

Illicit drugs such as heroin are often adulterated (cut) with other substances to either enhance or diminish the drug effects and to increase the weight and volume of the drug, thus increasing the dealer's profits. Many different substances are used to cut heroin. Some of the more common non-opiate cutting agents with pharmaceutical effect encountered by the Controlled Substances Section of PRIFS were: caffeine, procaine, cocaine, quinine, lidocaine, and the most frequently detected substance, xylazine. In 2007, a total of 663 suspected street heroin items (or exhibits) were analyzed qualitatively by gas chromatography/mass spectrometry (GC/MS). Heroin was present in 92% (613) of the total items. Of the remaining 8% (50) of the items, 40 (80%) items had xylazine as the main drug. These 40 items represent 6% of the total 663 analyzed items (Table 1).

From the 613 positive heroin items, heroin was identified in 43% (265) as the only drug. In 57% (348) of the items heroin was found in combination with other drugs. The most common heroin combinations were heroin/xylazine (36%), heroin/caffeine (22%), heroin/xylazine/caffeine (13%), heroin/quinine (9%), heroin/cocaine (4%), heroin/xylazine/cocaine (3%), heroin/xylazine/quinine (3%) and other drugs combinations (Table 2). Of the 348 heroin items, 199 (57%) had xylazine as an adulterant. Figure 1 shows a typical chromatogram result obtained from a street sample folded in a sheet of aluminum foil (Figure 2).
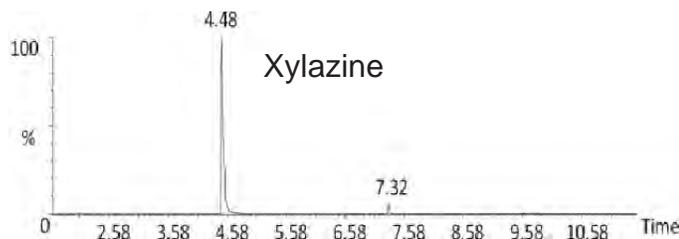


Figure 1. Chromatogram showing a typical street heroin item received by the Controlled Substances Section of PRIFS.

Table 1. Number and percentage of items without heroin, 2007 PRIFS

| Table 1. Number and percentage of items without heroin, 2007 PRIFS | Total | Percentage |
|---|---|---|
| Xylazine | 32 | 64 |
| Xylazine/Caffeine | 1 | 2 |
| Xylazine/Caffeine/Cocaine | 1 | 2 |
| Xylazine/Caffeine/Cocaine/Lidocaine | 1 | 2 |
| Xylazine/Cocaine | 1 | 2 |
| Xylazine/Quinine | 4 | 8 |
| Caffeine | 3 | 6 |
| Caffeine/Lidocaine | 1 | 2 |
| Caffeine/Quinine | 1 | 2 |
| Quinine | 5 | 10 |
| Total | 50 | 100 |

Table 2. Most Frequently Identified Heroin Combinations Number and percentage of identified heroin combinations, 2007 PRIFS

| Table 2. Most Frequently Identified Heroin Combinations Number and percentage of identified heroin combinations, 2007 PRIFS | Total | Percentage |
|---|---|---|
| Heroin/Xylazine | 125 | 36 |
| Heroin/Xylazine/Caffeine | 45 | 13 |
| Heroin/Xylazine/Cocaine | 9 | 3 |
| Heroin/Xylazine/Quinine | 9 | 3 |
| Heroin/Xylazine/Lidocaine/Procaine | 3 | 1 |
| Heroin/Xylazine/Caffeine/Lidocaine | 2 | 1 |
| Heroin/Xylazine/Caffeine/Procaine | 2 | 1 |
| Heroin/Xylazine/Other combinations | 4 | 1 |
| Heroin/Caffeine | 75 | 22 |
| Heroin/Caffeine/Quinine | 5 | 1 |
| Herion/Caffeine/Lidocaine | 3 | 1 |
| Heroin/Quinine | 33 | 9 |
| Heroin/Cocaine | 13 | 4 |
| Heroin/Cocaine/Other combinations | 4 | 1 |
| Heroin/Lidocaine | 4 | 1 |
| Heroin/Procaine | 4 | 1 |

The results of this statistical information show that xylazine is the main adulterant of the street heroin in Puerto Rico. Xylazine not only was found as an adulterant of heroin but also was found as the only component or in combination with other drugs. Xylazine may be fatal when used in combination with heroin or with other drugs. The combination of heroin and xylazine can elicit synergistic effects. Literature shows some similar pharmacologic effects between xylazine and heroin. Further studies are suggested to increase the knowledge and understanding of this emerging drug as an adulterant of heroin.

**Xylazine, Heroin, Adulterants**

## K46  Validation of a Method for the Determination of Opiates and Methadone in Hair

*Karen S. Scott, PhD\*, Department of Forensic Medicine and Science, University of Glasgow, University Place, Glasgow, G12 8QQ, UNITED KINGDOM*

After attending this presentation, attendees will have an insight into the methodology used to develop and validate a forensic toxicology method for hair analysis of opiates and methadone to IEC/ISO 17025:2005 standards within an accredited laboratory.

This presentation will impact the forensic community by providing laboratories considering obtaining accreditation with an insight into the methodology required for method validation.

The purpose of this study was to develop and validate a procedure for the determination of morphine, 6-acetylmorphine, codeine, dihydrocodeine, methadone, and EDDP in hair. Deuterated internal standard mixture and 0.1M HCl were added to 20 mg of specimen, control or spiked blank hair and sonicated for 1 h. The analytes were then extracted by solid-phase and derivatized with BSTFA + 1% TMS prior to GC-MS-SIM analysis. The limits of quantitation were <100 pg/mg for all drugs and the limits of detection <50 pg/mg. The intra-day and inter-day precisions of the assay were determined at 500 ng/mg and 2000 ng/mg and were <10% for all drugs.

An evaluation of the suitability of internal and external control samples was carried out throughout the validation process. Internal and external controls consisted of either spiked blank hair samples or pooled positive hair samples. The validation process found the controls to be effective and laboratory methodology was amended for their inclusion in all subsequent batch analyses. The validation data demonstrate that the method for the analysis of opiates and methadone in hair is sufficiently reproducible, robust and sensitive to carry out routine analysis within an IEC/ISO 17025 accredited laboratory.

**Hair Analysis, Opioids, ISO 17025**

## K47  Postmortem Analysis of Cocaine, Benzodiazepines, Opiates, and SSRIs in Hair

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The goal of this study is to compare the occurrence of drugs in hair with findings in corresponding femoral blood specimens from several medicolegal death investigations.

This presentation will impact the forensic community by explaining how hair specimens can provide a unique avenue of study in cases of severe decomposition, embalming, or chronic fetal drug exposure.

The purpose of this study was to compare the occurrence of drugs in hair with findings in corresponding femoral blood specimens from several medicolegal death investigations. Hair specimens can provide a unique avenue of study in cases of severe decomposition, embalming, or chronic fetal drug exposure. Extracts from hair can be applied to ELISA, LC-MS, and GC-MS analytical techniques for drug screening and confirmation. In the present study, hair extracts were evaluated by ELISAs according to the shown cut-off values, and also assessed by LC-MS/MS library matching. These results were compared to the drugs found in the corresponding femoral blood collected at autopsy.

| ELISA cut-off values (pg/mg) | |
| --- | --- |
| Carisoprodol | 1000 |
| Cocaine | 500 |
| Benzos | 200 |
| Fentanyl | 20 |
| Methadone | 200 |
| Opiates | 200 |
| Oxycodone | 300 |
| Tramadol | 1000 |
| Propoxyphene | 200 |
| Amphetamine | 500 |

Parent drugs, as well as some of the metabolites, accumulate within the hair cortex as the follicle grows. This evidence of drug use is stably incorporated into the hair, and does not usually diminish with standard hygienic practices. It is possible for drugs to associate with hair indirectly through sweat and sebum secretions, as well as contact with drug powder or smoke. However, when proper external decontamination is applied in combination with the presence of biologically derived drug metabolites, issues surrounding lingering external contamination diminish.

Hair must be broken down to release the drugs trapped within the protein structures, which can often destroy drug evidence or obscure the interpretation of parent/metabolite ratios. Current methods involve the use of mechanical or harsh chemical treatments to degrade hair. Mechanical disintegration requires specialized equipment, and strongly acidic or alkaline chemical treatments can further degrade the compounds of interest. This work describes methods for analyzing the drugs in hair with minimal effort, without destroying evidence.

Ten postmortem hair specimens were chosen for evaluation, which represent a wide variety of putative cocaine, benzodiazepine, SSRI, opiate, fentanyl, and methadone combinations based on prescribed and historical drug use. The hair samples (10-20 mg) were washed, dried, and weighed before further analysis to remove external contaminants. Extracts for ELISA analysis were prepared by incubating hair specimens in phosphate buffer for 2 hours at 60ºC. These formulations were diluted 1:5 in phosphate buffer before analysis. For LC-MS/MS analysis, the specimens were immersed in a 200 mM dithiothreitol solution, supplemented with a 100 ng/mL mepivacaine internal standard. Following 2 hours incubation, the extract was diluted with a 0.2% formic acid/20% acetonitrile solution, then filtered and stored in autosampler injection vials. The yield of drugs produced by this method was sufficient to apply towards LC-MS/MS screening library and confirmation assays.

The results obtained from these methods correlated well with drugs found in femoral blood extracts, where the cause of death was usually attributed to combined drug toxicities. The presence of other drugs in hair that were not present at the time of death illustrated a history of use. Additionally, the presence of other alkaloids and adulterants were found, such as noscapine and lidocaine, which supports the evidence of illicit sources of opiates and cocaine. Such contaminants are usually found in clandestine compositions, while pharmaceutical companies provide cleaner preparations. These detection procedures are technically feasible and efficient methods for releasing drugs trapped within hair for the purposes of forensic toxicology analysis, which aids in describing a pharmacological history of the decedent.

**Postmortem, Hair, Toxicology**

## K48 Evaluation of Alcohol Markers in Postmortem Hair and Blood: Comparison Between Ethyl Glucuronide, Ethylsulphate, and CDT

*Robert Kronstrand, PhD\*, National Board of Forensic Medicine, Department of Forensic Toxicology, Artillerigatan 12, Linkoping, SE 587 58, SWEDEN; and Henrik Druid, MD, PhD, Department of Forensic Medicine, Karolinska Institutet, Retzius v. 3, Stockholm, SE-171 77, SWEDEN*

After attending this presentation, attendees will understand the incorporation of EtG and EtS in hair as well as the use of CDT measurements to diagnose chronic alcohol use in the deceased.

This presentation will impact the forensic community by presenting new analytical technique and data on new markers of alcohol abuse.

Forensic medicine primarily deals with investigation of apparent or suspected unnatural deaths. Analysis of alcohol and the interpretations of its influence may be crucial in the investigation of traffic accidents, suicides, and homicides, but also in other cases. Since chronic alcoholism is one of several underlying diagnosis that can explain the cause of death in obscure cases, identification of heavy alcohol abuse is an important issue in forensic medicine. However, markers of alcohol over-consumption have previously been criticized for not having sufficient specificity. As a result of this, identification of alternative markers has been encouraged. Measurement of CDT and phosphatidyl ethanol has previously been evaluated in postmortem population and recently there has been interest in direct markers of ethanol consumption. Ethyl glucuronide (EtG) and ethyl sulphate (EtS) are exclusively formed after ethanol exposure, and is incorporated in hair. This study was performed to provide diagnostic improvement of alcohol abuse in forensic medicine by comparing the findings of EtG and EtS in hair with that of blood CDT as well as with the medical history of the deceased.

The study was approved by the Regional Research Ethics Committee in Linköping (#M47-08). The study material was collected at the Departments of Forensic Medicine in Stockholm, Linköping and Lund. Forensic nurses interviewed the relatives of deceased persons and retrieved information from medical journals and police reports to investigate the alcohol history of the persons. From each subject, samples of hair and blood were collected and analyzed for EtG, EtS and CDT. Based on the background information, the subjects were divided into five groups: persons with no or limited alcohol intake (N=5), occasional drinkers (N=4), moderate drinkers (N=8), alcohol abusers (N=11), and excessive alcohol abusers (N=15). EtG and EtS in hair were measured by ultra performance liquid chromatography/electrospray tandem mass spectrometry (UPLC/ESI-MS/MS) on a 3 cm portion of the hair. These results were compared with reported alcohol consumption, and with blood levels of CDT determined by HPLC (see Table for mean values). In total, 43 deceased subjects were included in the study. A correlation was found between EtG and EtS levels in hair. EtG correlation with background information was probably blurred by uncertain background information, but when only cases with a high degree of reliability concerning alcohol intake information were included differences between the groups became more pronounced (See Figure). Only 19 of the blood samples could be analyzed for CDT owing to problems obtaining "postmortem" serum resulting in matrix interferences in the HPLC chromatogram. In eight of the cases CDT levels above 2% indicated overconsumption (see Figure insert). A low correlation ($R^2$= 0.28) between EtG in hair and CDT in serum was found. One explanation for this might be that the time windows are different, CDT being elevated 4-6 weeks after cessation of drinking whereas EtG in hair had longer detection time because of the 3-cm hair length analyzed. Using a 30 pg/mg cut-off, all but three cases should have been diagnosed as over consumption, including two of the cases with limited consumption reported. In conclusion, EtG and EtS showed similar trends and no preference for the other could be discerned. CDT was difficult to analyze in more than half of the samples. Further studies including a larger number of study objects and more reliable background information are required before a final cut-off value can be established.

| Group | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| EtG (mean pg/mg) | 361 | 398 | 493 | 541 | 907 |
| EtS (mean pg/mg) | 220 | 618 | 1054 | 975 | 2604 |
| CDT (mean %) | 0.7 | 1.5 | 1.3 | 1.7 | 3.3 |
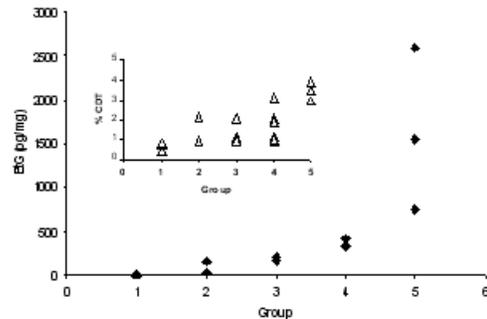


**Ethyl Glucuronide, Ethyl Sulphate, CDT**

## K49 Forensic Toxicology Findings in Blood and Urine From Female Victims of Alleged Sexual Assault

*Fredrik C. Kugelberg, PhD\*, National Board of Legal Medicine, Department of Forensic Toxicology, Artillerigatan 12, Linkoping, SE-587 58, SWEDEN*

After attending this presentation, attendees will acquire up-to-date information about the occurrence of ethanol and other drugs in blood and urine from female victims of alleged sexual assault in Sweden.

This presentation will impact the forensic community by providing data on the most common drugs found in female victims of alleged sexual assault in Sweden and will also aid forensic toxicologists in the interpretation of such cases.

Cases of alleged drug facilitated sexual assault (DFSA) have been increasingly reported in forensic science and medical journals since the 1980s. In news media the term "date rape" is often seen to describe such cases, although toxicologists and medical practitioners prefer the acronym DFSA, which implies use of a chemical agent to facilitate non-consensual sexual contact. The prevalence and the types of drugs encountered during investigations of alleged sexual assault are likely to differ between countries depending on social norms and the availability and popularity of recreational drugs. Ethanol, either alone or together with other drugs, has been a common finding in many previous surveys of DFSA from the United States and United Kingdom. Indeed, some victims suspect that their drinks had been spiked with a drug to explain the condition they found themselves in e.g., sudden incapacitation, sedation and subsequent drug-induced amnesia. Fast acting sedative-hypnotics such as gamma-hydroxybyturate (GHB) and flunitrazepam have frequently been associated with so called "drink spiking". However, compelling evidence that a person was incapacitated by voluntary or involuntary consumption of alcohol and/or drugs is not easy to obtain from results of toxicological analysis.

The population of Sweden is just over 9 million and forensic toxicology is done at one central laboratory. The results of toxicological analyses for ethanol and drugs are entered into a database (ToxBase)

along with age and gender of the individuals concerned. All cases registered between 2003 and 2007 where indications existed that a female had been subjected to some form of sexual assault were included in this study (N = 1,806). In many cases the police made a special request for toxicological analysis for certain substances because intoxication and "date rape" already formed a part of the investigation. During examination of the victim by a physician, which often occurred several hours after the event, specimens of blood and if possible urine were taken and sent for toxicological analysis. This entailed a broad screening by use of immunoassay methods (EMIT/CEDIA) on urine if available or on blood after protein precipitation. All positive results from screening were verified by more specific methods, involving isotope dilution gas chromatography-mass spectrometry. The analysis of a large number of prescription drugs was done by capillary column gas chromatography with a nitrogen-phosphorous detector. The concentrations of ethanol in blood and urine were determined by headspace gas chromatography and values below 0.1 g/L were reported as negative.

The present series of 1,806 cases consisted of blood only in 207 cases (11.5%), both blood and urine in 1,431 cases (78.7%), only urine in 170 cases (9.4%), and hair in 8 cases (0.4%). Accordingly, blood samples were available for toxicological analysis in 90% (N = 1,628) of cases of alleged sexual assault over the 5-year period. The number of cases of DFSA was highest during the warmer summer months (June-September) and the mean age of victims was 24 y (median 20 y), with ~60% being between 15 and 25 years. In 559 cases (31%) ethanol and drugs were negative, 772 cases (43% of total) contained ethanol alone, 215 cases (12%) contained ethanol with at least one other drug and 262 cases (15%) contained either licit (prescription) or illicit drugs (amphetamine, ecstasy, cannabis, cocaine, GHB). The mean, median and highest blood concentrations of ethanol (N = 806) were 1.24 g/L, 1.19 g/L and 3.7 g/L, respectively. Higher blood-ethanol was associated with increasing age of victims (r = 0.365, p<0.001). Amphetamine (N = 86) and tetrahydrocannabinol (N = 100) were the most common illicit drugs identified in blood at mean (median) concentrations of 0.22 (0.10) mg/L and 0.0012 (0.0006) mg/L, respectively. A wide spectrum of prescription drugs was identified in these cases, including sedatives such as diazepam (N = 88), alprazolam (N = 55) and zopiclone (N = 35), but mostly at therapeutic concentrations. Among non-sedatives, antidepressants (SSRIs), paracetamol and opiates (codeine and tramadol) were the major findings. These drugs might have been used to treat a legitimate medical condition and have nothing to do with chemical submission or DFSA. However, finding high therapeutic concentrations of drugs with short half-lives, such as zolpidem and zopiclone, makes it more likely that sedation and DFSA was involved.

Ethanol was by far the dominant psychoactive substance identified in blood and urine samples in these DFSA cases and this agree well with studies from other countries (e.g. USA and UK). T he high average blood-ethanol concentration of 1.24 g/L at time of sampling verifies that considerable amounts of alcohol had been consumed, especially if a back extrapolation of the concentration to time of attack is made. Relating the blood-concentrations of ethanol and/or drugs to the degree of incapacitation and helplessness of the victim is fraught with difficulties.

**Drugs, Ethanol, Sexual Assault**

# K50 Validation of a Color Test for Gamma-Hydroxybutyrate and Gamma-Butyrolactone

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After attending this presentation, attendees will have a better understanding of the ferric hydroxamate color test for the detection of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL).

This presentation will impact the forensic science community by serving to provide a validated effective screening/presumptive color test for the detection of GHB in evidential samples.

GHB is one of the most widely-used drugs for drink adulteration in "date-rape" cases. Several test kits have been introduced to detect GHB in drinks; however, presently there is no single effective color test available for use by forensic drug laboratories to screen evidential submissions for GHB and GBL. Previously, Michalik et al.[1] described the creation of a new colorimetric spot reagent for the detection of GHB and GBL. This colorimetric spot reagent was an adaptation of the ferric-hydroxamate test for lactones.[2] It is a simple test that requires little sample preparation, and takes just a few seconds to accomplish. The test is able to detect 1 mg/mL of GHB or GBL in solid-dosage submissions as well as various matrices such as soft drinks and alcoholic beverages. This presentation will highlight the data and tests conducted to validate the hydroxamate colorimetric spot test for the detection of GHB and GBL in different matrices.

This work was conducted in order to determine how well the ferric hydroxamate test would be able to detect GHB and GBL in a variety of matrices including beverages. The procedure is a four-step procedure: (1) addition of 1 drop of concentrated $H_2SO_4$, (2) 3 drops of 0.5M hydroxylamine HCl in 95% ethanol/6M NaOH, (3) 1 drop of concentrated HCl, (4) 1 drop of 5% $FeCl_3$. Treatment of solutions of GHB and/or GBL with the reagents in this successive procedure produces a deep magenta color immediately. The magenta color formed with a positive reaction was distinctly different from the color of a negative reaction, which produces a brown precipitate or a ferric chloride solution, which was light yellow in color. Using commercially purchased synthetic GHB in pure deionized water, the hyrdroxamate color test gave a positive magenta color response down to 1 mg/mL. No false negatives were observed. All tests were conducted on the matrix, a water blank (deionized $H_2O$, concentrated $H_2SO_4$, and reagents), reagent blank (reagents only), and a matrix blank (matrix, reagents, and concentrated $H_2SO_4$). Potential interferents and water gave a similar response. Experiments with beverages containing GHB focused on allowing spiked and unspiked beverages to be compared directly.

Matrices tested included water (tap and bottled), coffee (regular and decaf), cranberry juice, orange juice, sprite, coca-cola, lime juice, lemon juice, pineapple juice, an energy drink, mouthwash, and various alcoholic beverages including several different wines, beers, and liquors. Analytes tested were GHB, GBL, 1,2-butanediol, 1,3-butanediol, 1-4-butanediol, 2,3-butanediol, beta-butyrolactone, gamma-valerolactone, caprolactone, dihydrocoumarin, dextromethorphan hydrobromide, caffeine, ephedrine, papaverine, cocaine, diazepam, and methamphetamine. It should be noted that the only other compounds tested that gave a false positive were other lactones (gamma-valerolactone caprolactone, dihydrocoumarin, and betabutyrolactone). This is to be expected since the test is specific for lactones, but is not of concern since these compounds are normally not encountered in GHB submissions. Of particular note, the test is negative for alcoholic beverages tested except for the red wines which were difficult to interpret due to the color of the solution.

The color formation with the ferric-hydroxamate reagents constitutes a highly specific screening test for GHB and/or GBL in a variety of matrices normally encountered in a forensic environment. The low cost of the reagents and their apparent reliability suggest that the test would be a useful screening tool for forensic scientists in the crime laboratory as well as officials investigating rape cases.

**References:**
1. K Michalik and TA Brettell, Abstract #B71, 60th Annual Meeting of the AAFS, Washington, DC, February 22, 2008.
   WC Alston and K Ng Forensic Sci. Int. 2002; 126(2002):114-117.
**Forensic Science, GHB, GBL**

# K51 Quantitative Mass Spectrometric Imaging of Drugs of Abuse in Postmortem Human Brain Tissue

*Richard F. Reich, MS\*, University of Florida, PO Box 14044, Gainesville, FL 32604*

After attending this presentation, attendees will understand how to perform direct detection and quantitation of drugs of abuse in intact tissues by using deuterated internal standards and matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS$^n$).

Direct quantitative mass spectrometric imaging of intact tissue will impact the forensic science community by providing an alternative approach to conventional drug analysis in tissue, which typically involves tissue homogenization, resulting in loss of histological information for drug distribution.

The ability to measure the regional distribution and concentration of drugs of abuse and their metabolites in postmortem brain tissue of chronic human drug users would be an invaluable tool in determining the pharmacological and toxicological actions of the drug of abuse in the human brain. Conventional drug analysis in tissue involves homogenate preparation, followed by extraction and/or derivatization. The extracts are then analyzed by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). Sample pretreatments are known to introduce variation in detection. The preparation of tissue homogenate precludes the opportunity to acquire detailed histological information for drug distribution. Mass spectral imaging using MALDI-MS$^n$ provides an alternative approach for the quantitative imaging of drugs of abuse in human brain tissue, while keeping the physical features of the autopsied brain intact.

Tissue samples were excised from the nucleus accumbens (a dopamine-rich area of the striatum) and were snap frozen in liquid nitrogen and stored at -80ºC. The drug of abuse and its deuterated analog (internal standard) were spiked onto 20-μm tissue slices using a micropipet. Using an artistic airbrush, MALDI matrix was applied to the tissue. The distribution of the drug of abuse in tissue was imaged using a linear ion trap with intermediate-pressure MALDI source. A MS$^n$ isolation window was selected to include the [M+H]$^+$ ions of both the drug of abuse and the internal standard. An average of ten laser shots per scan was used to produce mass spectra.

Experiments show that ratioing the peak intensities of the analyte and a deuterated internal standard reduces shot-to-shot variability, which is due, in part, to nonhomogeneous crystallization of the matrix on tissue. The MALDI matrix for each drug analysis was chosen based on its ability to ionize the analyte efficiently and to minimize interfering ions. 2,5-Dihydroxybenzoic acid was chosen for the analysis of cocaine and its metabolites; α-cyano-4-hydroxycinnamic acid was determined to be the optimal matrix for the analysis of 6-monoacetylmorphine and morphine.

Brain tissue is a complex sample environment containing a multitude of endogenous lipids and other species that can act as interferants. MS$^n$ methods were developed to increase the selectivity and sensitivity for the target drug analytes in brain tissue. MS$^n$ parameters were optimized for the [M+H]$^+$ ions of cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, 6-monoacetylmorphine, morphine, and the corresponding trideuterated analogs of these species. Instrument software allows for only one isolation window in MS$^n$ experiments, isolating one parent mass (or range of masses) for collision-induced dissociation (CID). This means that MS$^n$ of the target ions of the analyte and internal standard would typically be performed with two separate MS$^n$ experiments. This would increase the response variability and counteract the signal normalizing effects of using an internal standard. Using a 6-amu-wide isolation window centered at a mass-to-charge between the [M+H]$^+$ ions of the drug analyte and its deuterated analog allows for isolation and CID of both ions during a single MS$^n$ experiment. This single isolation method reduces the signal variability inherent with MALDI compared to isolating each ion individually with a 1-amu window (in two alternating MS$^n$ experiments). This method was used to detect and quantitatively image drugs of abuse and their metabolites in postmortem human brain tissue.

This study demonstrated that MS$^n$ increases selectivity, which is critical for differentiating analyte ions from matrix ions and endogenous compounds found in brain tissue. It was also shown that the use of internal standards corrects for signal variability in quantitative MALDI arising from inhomogeneous crystal formation, inconsistent sample preparation, and laser shot-to-shot variability. Using a single MS$^n$ experiment with a wide isolation window to isolate both analyte and internal standard target ions provided improved precision (10-20 times reduction in %RSD) for quantitative imaging studies compared to using two alternating MS$^n$ experiments that isolate the analyte and internal standard target ions separately.

**Mass Spectrometric Imaging, Drug Quantitation, Brain Tissue**

# K52 6-Monoacetylmorphine Confirmation by Liquid Chromatographic Tandem Mass Spectrometric Determination of 6-Monoacetylmorhine and Noscapine in Vitreous Humor

*Terry Danielson, PhD, Ashraf Mozayani, PhD, PharmD, Jeffrey P. Walterscheid, PhD\*, Shaohan Zhao, PhD, and Luis A. Sanchez, MD, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will learn of the great care required in the interpretation of low level determinations by liquid chromatographic tandem mass spectrometric (LC/MS/MS) methodologies.

This presentation will impact the forensic community by demonstrating how LC/MS/MS is an extremely sensitive technique and extraordinarily low levels of drugs are routinely detected. Interpretation at these extremely low levels is not straight-forward.

The objective of this work is to present a potential pitfall in modern tandem mass spectrometric methodologies. Tandem mass spectrometry is an extremely sensitive technique and extraordinarily low levels of drugs are routinely detected. Interpretation of these extremely small amounts of drugs or metabolites is not straightforward. For example, substances with similar retention times and the same ion transitions can potentially result in false positives, as has been observed in the liquid chromatographic tandem mass spectrometric (LC-MS/MS) analysis of succinylcholine and venlafaxine. These instances illustrate a potential limitation of typical multiple reaction monitoring (MRM) techniques in qualitative confirmations of these non-routine analytes.

It has been observed that a similar deficiency may occur in the LC-MS/MS identification of 6-monoacetylmorphine (6-MAM), a commonly accepted marker of heroin abuse. On occasion, it has been observed LC-MS/MS peaks with retention times and ion transitions very similar to 6-MAM have appeared in specimens containing morphine but not anticipated to involve heroin.

The veracity of MRM identification of this "6-MAM" was examined by simultaneously determining the presence of 6-MAM and noscapine in vitreous humor. Noscapine is an alkaloidal substance, found in the opium poppy. It persists throughout the manufacture of heroin and it, and other similar alkaloids, have been proposed as urinary markers of heroin usage.

Morphine, 6-MAM, and noscapine was examined in vitreous humor from a series of twelve morphine-positive cases. Two of the cases were thought to not involve heroin, although 6-MAM had been putatively identified in stomach contents by LC-MS/MS. Morphine was confirmed in all of the vitreous specimens, 6-MAM in ten, and noscapine in eight. All of the noscapine-positive specimens also contained 6-MAM. Neither 6-MAM nor noscapine were detected in vitreous from the two cases not expected to involve heroin, although 6-MAM had previously been "detected" by LC-MS/MS in stomach contents.

Noscapine was employed as an alternate indicator of heroin use and suggest that 6-MAM might be falsely identified by typical MRM techniques. This suggests that LC-MS/MS identifications based on a small number of ion transitions are fallible, and that great care must be taken during the interpretation of detecting 6-MAM at low levels.

**6-Monoacetylmorphine, Noscapine, LC/MS/MS**

## K53 National, Regional, and Local Patterns of Drugs Seized by Law Enforcement and Analyzed by Crime Laboratories in the United States: 2004 - 2007

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After attending this presentation, attendees will better understand the complexity and geographical variation of the U.S. drug problem and will also recognize the contributions of forensic laboratories and scientists not only to drug law enforcement issues but also to providing key scientific data for drug policy initiatives. This presentation will focus on the "supply side" of the drug problem by addressing the issue of the distinct drugs seized by law enforcement agencies and analyzed by over 275 of our nation's crime laboratories.

The presentation will impact the forensic community by acknowledging the large contribution of crime laboratory forensic scientists. The presentation will also contribute to a clearer understanding of varying dimensions and components of drug trafficking and abuse of both licit and illicit drugs.

Our nation's drug problem consists of patterns of trafficking, consumption, and diversion of both licit controlled drugs and illicit drugs that vary across time and location. Data from DEA's National Forensic Laboratory Information System (NFLIS) will be presented to depict key issues concerning national, regional and local drug problems. State and local forensic laboratories analyze substances secured in law enforcement operations across the country and offer a valuable resource for monitoring and understanding drug abuse and trafficking, including the diversion of legally manufactured drugs into illegal markets. During the period January 2004 through December 2007, an estimated 7,227,531 drug items were analyzed by state and local laboratories in the United States. The number and percentage of the top five controlled prescription drugs and the top four illegal drugs analyzed during 2004-2007 will be presented at national and regional levels (see table below). The distribution of these drugs across state and metropolitan areas will also be examined.

| | National | | West Region | | Midwest Region | | NE Region | | South Region | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Illegal Drugs* | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** |
| Cocaine | 2,363,308 | 32.70 | 283,498 | 20.30 | 453,621 | 25.93 | 464,081 | 37.85 | 1,162,108 | 39.78 |
| Cannabis/THC | 2,371,585 | 32.81 | 324,365 | 23.22 | 792,232 | 45.03 | 367,070 | 29.93 | 887,915 | 30.39 |
| Methamphetamine | 848,495 | 11.74 | 512,157 | 36.7 | 122,488 | 7.27 | 6,127 | 0.50 | 207,723 | 7.11 |
| Heroin | 372,141 | 5.15 | 49,342 | 3.54 | 82,095 | 4.87 | 128,437 | 10.47 | 112,265 | 3.84 |
| *Prescription Drugs* | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** | *Number* | *%\** |
| Hydrocodone | 109,440 | 1.51 | 11,518 | 0.83 | 17,931 | 1.06 | 13,819 | 1.13 | 66,176 | 2.27 |
| Alprazolam | 108,734 | 1.50 | \*\* | \*\* | 21,124 | 1.25 | 14,746 | 1.20 | 66,791 | 2.29 |
| Oxycodone | 92,764 | 1.28 | 10,886 | 0.78 | 18,393 | 1.09 | 27,566 | 2.25 | 35,919 | 1.23 |
| Methadone | 33,882 | 0.47 | 4,767 | 0.34 | 5,365 | 0.32 | 8,564 | 0.70 | 15,197 | 0.52 |
| Clonazepam | 29,426 | 0.41 | 2,872 | 0.21 | 5,489 | 0.39 | 8,601 | 0.70 | 11,464 | 0.39 |

\* Percent columns represent percent of estimated total drug items analyzed in the period January 2004 - December 2007

\*\* Estimates for this drug do not meet standards of precision & reliability - too few laboratories reported this specific drug

Highlighted findings will include the prevalence of drugs seized and analyzed with special emphasis on controlled drugs such as opioid analgesics and benzodiazepines. Geographic Information System (GIS) generated maps will be used to display levels of seized drugs identified based on the "county of seizure" for a representative state from each of the census regions. The distribution of major drug categories across states as well as drugs identified in strategic locations will also be presented. The integration of GIS functionality for data exploration and display further enhances the importance of the NFLIS data as an informational resource for drug policy and drug control agencies by providing timely information on drug trafficking and abuse spatial patterns across the United States.

**Drug Seizures, Drug Database, Geographic Information System (GIS) Display**

## K54 A Nuclear Magnetic Resonance (NMR) Based Study of Urine Samples Containing Drug of Abuse: Scope and Limitations of the Technique

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After attending this presentation, attendees will learn about the use of NMR spectroscopy in drug of abuse detection.

This presentation will impact the forensic science community by exploring a new spectroscopic technique for the analysis of forensic samples.

Testing for substances of abuse in urine has great forensic relevance. The need for testing arises in many different situations: identifying drugs of abuse, supporting or denying a person's statement that they have or have not taken drugs, or determining what drug may have caused an overdose. In the following presentation, the advantages and limitations of using nuclear magnetic resonance (NMR) spectroscopy for the identification of substances of abuse in urine is

explored. Opioids were chosen for analysis as federal drug testing in urine mandates a higher cutoff level than other substances of abuse. NMR spectroscopy is the method chosen for the analysis on the basis of many advantages: it allows positive identifications of chemically different species (very similar substrates can be usually identified); very little sample preparation or operator training is needed; and, spectra could be gathered in very short times.

Codeine, morphine, and oxycodone were used in this study. Initially, these compounds were dissolved in an artificial urine solution of ten components formulated to model the NMR spectrum of real urine and NMR spectra were recorded. Later, real urine and forensic samples from deceased patients were used in the investigation.

From preliminary data, NMR spectroscopy has proven to be a novel, feasible, and useful technique for the study of opioids in urine samples. The three opioids, which present very similar structures, could be distinguished from one another in both water and artificial urine. Moreover, all three drugs could be identified at a concentration of 2000 ng/ml, equal to the federal cutoff limit given by the United States Department of Health and Human Services. This was easily done with a simple analysis of chemical shift differences. These characteristic peaks were observed at low concentrations suitable for drug testing. These peaks, arising from two protons on the phenyl group of phenanthrene opioids, were found between 6 and 7 ppm. For morphine, the difference in frequency was near 41 Hz while for codeine is near 71 Hz. For oxycodone, the difference in frequency is near 43 Hz. The ease of use NMR instrumentation, speed of analysis, as well as the small sample amount needed and the fact that is a non-destructive technique render NMR spectroscopy an advantage over current forensic methods used to analyze substance of abuse in urine.

**NMR, Opioids, Urine**

## K55 Sample Preparation of Cannabiniods in Urine Using Dispersive Solid Phase Extraction and Clean Up

*Jack Cochran, BS\*, and Kristi Sellers, BS, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823*

After attending this presentation, attendees will understand how to increase sample throughput using a simplified sample clean up method for analyzing cannabinoids in urine as well as understand how to derivatize and analyze cannabinoids by GC-MS.

The sample preparation and analysis methodology discussed will impact the forensic community by providing an alternate means of processing and analyzing cannabinoids compared to current sample preparation and analysis methodologies.

The main psychoactive component in marijuana, $\Delta^9$–tetrahydrocannabinol ($\Delta^9$–THC), is quickly absorbed and metabolized to 11-hydroxy-$\Delta^9$-tetrahydrocannabinol (hydroxy-THC), an active metabolite. The hydroxy-THC is further metabolized (rapidly) to 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol (carboxy-THC), an inactive metabolite commonly found in urine, blood, hair, and other tissues. GC-MS (Gas Chromatography-Mass Spectrometry) often is used for confirming and quantifying $\Delta^9$–THC and carboxy-THC. However, GC-MS methods require time-consuming steps like sample clean up to obtain acceptable chromatography. Using a dispersive solid phase extraction and clean up technique saves time without sacrificing reproducibility and sensitivity.

This study included developing a sample clean up method for analyzing cannabinoids in urine using a dispersive solid phase extraction and clean up method (dSPE). The dSPE method employs a quick extraction followed by a cleanup of the sample. Small polypropylene centrifuge tubes are prefilled with precise weights of $M_gSO_4$ and SPE (solid phase extraction) adsorbents to remove excess water and unwanted contaminants from the samples. After agitation and centrifugation, the cleaned extracts are ready for further processing. Samples may be derivatized, pH adjusted to protect sensitive compounds and/or solvent-exchanged to improve analysis by GC-MS. Internal standards can also be added. The samples are then ready for analysis by GC-MS. Also, dSPE and sample clean up process can be used for HPLC-MS (high performance liquid chromatography-mass spectrometry) applications.

A reproducible, quantitative GC-MS method for analyzing cleaned-up, derivatized cannabinoids in urine was developed. Two goals were the focus in this study: (1) to reduce sample clean up time for cannabinoids in urine, and (2) to provide a reliable and reproducible sample preparation method for quantification data in the low ng range (< 10ng). Compounds analyzed for were $\Delta^9$–THC and carboxy-THC. The internal standard used was deuterated THC. Derivatizing reagents experimented with included a silylation reagent and an acylation reagent. The instrument used was a Shimadzu GC-MS.

Results showed that the proper dispersive solid phase extraction and sample clean up method coupled with the proper derivatization reagent produced reproducible data with linearity across a broad range of concentrations. The limit of detection (LOD) reached was as low as 5ng on-column, and sample preparation time was reduced. The use of GC-MS allowed for identification of derivatized cannabinoids, particularly $\Delta^9$–THC and carboxy-THC, relative to their unique mass spectra. Analysis time was kept under 10 minutes since run conditions were optimized.

In conclusion, the methods developed in this study can benefit analysts by providing a simple and short extraction and clean-up procedure, by providing a reproducible derivatization procedure and by providing reduced analysis times using GC-MS for cannabinoids in urine.

**Sample Preparation, GC-MS, THC**

## K56 A Fast GC/MS Method for the Analysis of Common Selective Serotonin Reuptake Inhibitors

*Sumandeep Rana, MS\*, Wayne B. Ross, MCLS, and Victor P. Uralets, PhD, Redwood Toxicology Laboratory, 3650 Westwind Boulevard, Santa Rosa, CA 95403*

After attending this presentation, attendees will learn about a validated, fast method for routine analysis of common selective serotonin reuptake inhibitors (SSRI's) in human urine.

This presentation will impact the forensic community by demonstrating how choosing the right combination of column and carrier gas results in significant improvement in analytical procedures and improves throughput in a routine testing laboratory.

SSRI's included in this method are: fluoxetine, norfluoxetine (fluoxetine metabolite), sertraline, norsertraline (sertraline metabolite), citalopram, and paroxetine. Deuterated paroxetine (paroxetine-D6) was used as the internal standard.

Urine samples were hydrolyzed using β-glucuronidase from *Escherichia coli,* centrifuged for 5 minutes and then approximately 1 gram of a salt mixture (sodium chloride, sodium carbonate and sodium bicarbonate, 6:1:1 by weight) was added. The alkalinized urine specimens were extracted using liquid-liquid extraction with heptane/ dichloromethane/ dichloroethane/ isopropyl alcohol (10:5:5:1). The organic upper organic layer was separated and dried under air at $40^\circ$C. The dried sample extracts were derivatized for 10 min at $65^\circ$C with MSTFA/ammonium iodide/ ethanethiol reagent (50 mg/25 mL/75 µL).

GC/MS analysis was performed in electron ionization mode by selective ion monitoring (EI - SIM) using a single quadrupole mass spectrometer with inert ion source. A 230 volt GC oven was used to enable fast temperature programming and hydrogen was used a carrier gas. Separation was performed on a narrow bore column (10 m X 0.15 mm i.d.). All analytes were eluted within 4.5 minutes with Injection-to-injection analytical run time of 7.5 minutes. Three ions for each analyte; paroxetine (249.1, 264.1, 401.2), Fluoxetine (219.0, 262.2, 381.2), norfluoxetine (174.1, 320.1, 439.2), sertraline (274.0, 276.0, 377.1), norsertraline (274.0, 276.0, 320.0), citalopram (238.1, 324.1, 208.1), and two ions for the internal standard; paroxetine- D6 (252.1, 270.2) were monitored.

The procedure was applied to authentic urine specimens and the results showed that hydrolysis is essential to the optimum recovery of most analytes. All analytes were successfully detected in the 4.5 minute run time utilized. The limit of detection for all analytes was 100 ng/mL except citalopram, for which it was 50 ng/mL. The limit of quantitation for citalopram and paroxetine was 100 ng/mL and for all other analytes it was 150 ng/mL. Precision was within 6% and quantitative accuracy was over 94% for all analytes. The method was linear up to 20,000 ng/mL for paroxetine and upto 2000 ng/mL for all other analytes.

A fast and simple GC/MS method was developed for the routine analysis of common SSRI's in urine. This method can easily be used for other body fluids such as blood. The simple sample preparation, combined with short, narrow bore GC column and hydrogen as a carrier gas, drastically decreased sample turnaround time and increased throughput without compromising sensitivity or selectivity.

**SSRI's, GC/MS, Hydrolysis**

## K57 Evaluating the Presence and Dangers Associated With Heavy Metals in Commonly Encountered Consumer Products

*Lindsay A. Carbone, BS\*, Thomas H. Pritchett, MS, and Brian J. Gestring, MS, Cedar Crest College, 100 College Avenue, Allentown, PA 18104*

After attending this presentation, attendees will learn how to develop a new method for evaluating the presence of heavy metals and their transfer into human saliva.

Heavy metal contamination is a significant problem for consumer products imported from outside of the United States. This presentation will impact the forensic community by evaluating its regional prevalence and shedding light on a new method to evaluate the associated hazard.

Since the late 1970's lead levels in commercially available paints have been regulated in the United States by the Consumer Product Safety Commission (CPSC). Levels of lead above 600 ppm were banned on surface coatings, toys, and other items intended for children, and furniture. While this has been successful in regulating products made in this county, an unintended consequence of the global economy is that more of these regulated items are being produced outside of the U.S. For the most part, the CPSC relies on the foreign manufacturer to comply with these U.S. guidelines. Recently this self-regulation has resulted in the CPSC issuing numerous product recalls. Over 42 million toys have had to be removed from the market due to excessive lead paint contamination of the toy's surface. With clear published standards on lead levels, investigations into how these items were allowed on the market might well result in criminal charges.

While lead's toxicity is well established and its presence well regulated, other unregulated heavy metals found in paints as pigments or driers might also pose health risks. This preliminary study evaluated the presence of heavy metals in commonly encountered consumer products

that either by design or by chance; ends up in an individual's mouth. The most obvious candidate for this category is children's toys. While it is usually not safe, it is common for young children to put non-food items in their mouths. A number of children's toys including some that had been subjected to a CPSC lead paint recall were evaluated as part of this study. Samples were not strictly limited to children's toys but also included some items that adults might inadvertently put in their mouths. To this end, pencils, pens, and certain cosmetic items were also evaluated.

Samples evaluated in this study were first screened for the presence of heavy metals. Initially this was accomplished through the use of Scanning Electron Microscopy with Energy Dispersive Analysis (SEM EDS). This approach proved problematic so screening was then changed to a portable x-ray fluorescence (XRF) unit which allowed for rapid non-destructive real-time elemental analysis.

Currently there is an abundance of literature regarding lethal doses of heavy metals and their associated toxicities. What's lacking are any studies that demonstrate how much of these metals are transferred into human saliva, the matrix that these samples would be exposed to. To address this issue an experiment was designed where a fixed amount of solid lead was placed into a set volume of human saliva that was maintained in a conical tube at body temperature. The saliva was then sampled at intervals from 30 seconds up to 240 minutes and compared against a saliva blank maintained under the same conditions via atomic absorption spectroscopy (AAS). Statistically significant concentrations of lead were found after x minutes that remained reasonably consistent for the duration of the exposure. This was performed for each heavy metal that was being evaluated by AAS.

Samples that had positive screening results with the XRF were then evaluated through the saliva transfer test. A 1 cm square was excised from the sample and allowed to sit in human saliva maintained at body temperature for XX. Results were then compared with saliva blanks also maintained at temperature. The Excised samples were then removed from the saliva, mechanically broken down, and then replaced into the saliva again. After being allowed to sit for X minutes again, the saliva was revaluated for the presence of the heavy metal.

**Metals, Transfer, Saliva**

## K58 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will be better prepared to interpret postmortem pediatric cases when there are toxicological findings. Attendees will understand the fundamental differences between adults and children in respect to toxicological findings.

This presentation will impact the forensic science community by broadening and deepening the knowledge base around the role of toxicants in postmortem pediatric cases.

In this 10th 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

including pharmaco-toxicokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings.

This year's presentations will be by:

Dr. Andrew Baker, Chief Medical Examiner, Hennepin County Medical Examiner, Minneapolis, MN will present a case-specific issue involving the role of a toxicant in a pediatric death.

Dr. Dan Isenschmid, Chief Toxicologist, Wayne County Medical Examiner's Office, Detroit, MI will discuss a case highlighting the concept that "Children Are Not Small Adults" through discussion of single dose administration of oxycodone.

Dr. Karen Ross, Assistant Coroner and Pathologist, Jefferson Parish Forensic Center, Harvey, LA will discuss a case highlighting the continued concerns of over-the-counter (OTC) preparations and exposures in children. Despite the FDA's mandate to remove OTC infant preparations, it is doubtful that the use of such preparations in this population will stop. Recognition of safety concerns with these products in children far exceeds the relatively recent media attention given the subject.

Dr. Marina Stajic, Chief Toxicologist, Office of Medical Examiner, New York, NY, will bring forward an unusual case involving fentanyl in a child. As a potent opioid with a narrow therapeutic index, fentanyl represents both a highly effective pain medication and a potential significant contributor to toxic sequelae. The illicit exposure of children to this compound is of significant concern due to its toxicological properties.

**Pediatric, Toxicology, Postmortem**

## LW1 The Search, Recovery, and Identification of the Tsar's Children

*Michael D. Coble, PhD, AFDIL, 1413 Research Boulevard, Building 102, Rockville, MD 20850; Anthony B. Falsetti, PhD\*, CA Pound Human ID Lab, c/o Cancer/Genetics Research, PO Box 103615, Gainesville, FL 32610; and Peter Sarandinaki, SEARCH Foundation, Inc., 49 Lakeshore Drive, Oakland, NJ 07436*

After attending this presentation, attendees will learn about the identification processes applied to this ninety-year old mystery.

This presentation will impact the forensic science community by highlighting the importance of utilizing the multidisciplinary approach to identify Tsar Nicholas, his family, servants, and the "missing children."

In the spring of 1917, at the end of the February Revolution, Tsar Nicholas II abdicates the throne ending nearly 300 years of Romanov royal leadership and begins a journey across the countryside that eventually results in the death of his family at the hands of the Bolsheviks. On March 22, 1917, Tsar Nicholas is taken to the Alexander Palace, Tsarskoye Selo, south of Saint Petersburg where the rest of the family is staying. They are immediately arrested by the provisional government. In August 1917, the Kerensky government evacuated the Romanovs to Tobolsk in the Urals with the supposed goal to protect them from the rising tide of revolution. The Bolsheviks came into power in October of that year with the counter-revolution is beginning to grow. On July 17th, 1918, Bolshevik authorities led by Yakov Yurovsky, shot Tsar Nicholas II, his immediate family, and four servant members in the cellar of the Ipatiev House in Yekaterinburg, Russia. The family was told that they would be photographed to prove to the people that they were still alive and hopefully quell the growing White movement.

In 1991, the remains of nine (9) individuals were discovered on the Koptyaki Road, outside of Yekaterinburg by geophysicist Alexander Avdonin. Following the discovery, a group of scientists from Russia and the United States began the process of analyzing the remains. Many Academy experts including Drs. William R. Maples, Lowell Levine, Michael Baden, William Hamilton, Diane France, and William Goza, participated in those examinations and presentations of their findings have been made at previous AAFS meetings. In 1998, results of molecular analyses in the United States, Britain, and Russia demonstrated that the bones belonged to the Romanov's and their remains were buried in Saint Petersburg in the Peter & Paul Cathedral. Just prior to their internment, at least two members of the Academy traveled to Yekaterinburg to provide guidance to the Russians on their continued efforts to find the missing children.

In late September 2007, after nearly 15 years of searching, Russian archaeologists located the burial site of at least two additional individuals. These archaeologists armed with new interpretations of "Old Russian" provided by an archivist in Moscow redirected their efforts on the Koptyaki Road and found the bones approximately 70 meters from the site where the nine bodies were discovered in 1991. Over the span of several months various physical examinations of the remains were undertaken by Russians and Americans. All concluded that the skeletal remains, 44 fragments of bone, including teeth, represented at least two individuals, one a young female and the other, likely male and young. Evidence of postmortem change due to the passage of time, as well evidence of traumatic injury was also noted. Material evidence recovered by the archaeology team, led by Dr. Pogorelov, included time-appropriate projectiles and ceramics. Since no duplication of skeletal elements was seen between the two grave sites, and for the first time bone from a young male is present, it was thus concluded that these were additional individuals from a similar event who died during a nearly identical time period. Arrangements were made to once again attempt to recover DNA from suspect Romanov bone by several laboratories. The AFDIL, a Russian lab, directed by Dr. Evgeny Rogaev, and Dr. Walther Parson's laboratory in Austria were contacted and all agreed to participate. Bone was sampled from each individual and results demonstrated that, one was male, one a female, that they were Romanov's and they were likely siblings.

**Tsar Nicholas, Identifcation, Final Chapter**

## LW2 Persona of a Crime: L'Affaire Praslin From 1847 to 2009

*Jennie Meade, JD, MLS\*, The George Washington University Law Library, 716 20th Street, Northwest, Washington, DC 20052*

After attending this presentation, attendees will learn about the multiple manifestations of the infamous 1847 Paris society murder that has spanned more than a century and a half in France and America. Attendees will also understand the range of its influence, from the level of the French state to fiction and film.

This presentation will impact the forensic science community by illuminating the mix of effects, from government to forensic to artistic, resulting from L'Affaire Praslin.

In 2001, the George Washington University Law Library received a shipment of French antiquarian law books purchased at auction in London. One large lot contained a book marked simply "Procedure." In it was a large folding diagram of a bloody murder scene, showing a bedroom with furniture in disarray and blood madly spattered throughout from floor to furnishings. This was a map of the August 18, 1847, Paris murder of the Duchesse de Praslin by her husband, the Duc: the infamous "Affaire Praslin" which shocked France and the international community, jolted the French government off course, provided fodder for writers and filmmakers, and connected America with France in an unforeseen way. Unnoticed by the auction house in such a large lot, this unassuming tome, which also contained legal papers relating to the case, proved to be a rare addition to the library's French Collection and the catalyst for research into this officially unsolved crime whose aftershocks have reached into the 21st century.

The Duc de Praslin, scion of one of the ancient noble families of France, and his wife Fanny Sebastiani, the Duchesse, had seen their marriage progressively sink into desolation. One focus of their distress was Fanny's problematic relationship with their younger children, and the couple agreed to engage a new governess, Henriette Deluzy, to assume the role, along with the Duc, of caring for the children. Deluzy's immediate and sustained popularity with the children, as well as the Duc, incited a devouring envy in the emotionally-fragile Duchesse, who eventually effected Deluzy's dismissal amid accusations of adultery. A scant month later, the Duchesse was found in her bedchamber, beaten and stabbed to death during an evidently prolonged horrific struggle, after which the Duc was found in his quarters, having made attempts at washing bloodstained clothing and with charred fabric in his fireplace. Shortly thereafter, the Duc ingested arsenic and died before trial but not before concocting an insubstantial account of the event.

The murder astounded France. Yet the idea that a high-ranking noble, a relative and state colleague of King Louis-Philippe, could have committed a killing so depraved and shocking a killing served to bolster

the conception of the idle aristocracy's degeneracy prevalent in the French populace. The July Monarchy, already weak, fell by revolution in early 1848 with the Praslin murder as one of the immediate causes.

L'Affaire Praslin brought early celebrity to Auguste Amboise Tardieu, the gifted French forensic pathologist. Tardieu reconstructed the crime from hair and skin fragments on a pistol examined microscopically and from matching the Duchesse's head wounds to the size of the pistol butt. Tardieu's adept conjecture quashed the Duc's attempts at his own fabrication of events.

L'Affaire Praslin continued to thrive in various media long after its role in changing the course of French history. The intriguing and strange story inspired fictional treatments plus a major film. Nathaniel Hawthorne is believed to have modeled the character Miriam in *The Marble Faun* (1860) on the governess Deluzy. In 1938, award-winning American author Rachel Field published *All This and Heaven Too*, cinematized in 1940 as Warner Brothers' answer to *Gone with the Wind*, starring Bette Davis as the governess and Charles Boyer as the Duc.

Rachel Field's interest in the governess was not just academic: Deluzy was her great-aunt by marriage. Haunted by the crime (although exonerated of wrongdoing), the now-notorious "Mademoiselle D" had emigrated to America in 1849 where she married into a prominent New York family. Her new husband was clergyman Henry Martyn Field, brother of the eminent lawyer David Dudley Field. In Gramercy Park, Mrs. Field reigned for more than two decades as a cultivated and scintillating hostess over a salon which became a meeting place for writers, artists, and academics, including William Cullen Bryant, Harriet Beecher Stowe, Samuel Morse, and Fanny Kemble. Gradually, her identity as "Mademoiselle D" was forgotten and upon her death in 1875 she was eulogized as "one of the most distinguished women of New York."

The strange allure of l'Affaire Praslin continues. *France Magazine* chose to begin its 2006 profile of the French Collection at GW's Law Library with the map of the murder scene and the words: "It was a terrible crime…"

**Praslin, Murder, Deluzy**

# LW3 The Scientific Genius of Archimedes: How Do We Know That Much of It Was Real?

*Abraham T. Philip, MD\*, Onondaga County Medical Examiner, 100 Elizabeth Blackwell Street, Syracuse, NY 13210*

After attending this presentation, attendees will be made familiar with the life and work of Archimedes, who is generally regarded as the father of mathematics, calculus and applied mechanics. This presentation will also highlight techniques used to bring to life ancient texts long thought to be lost to humanity.

This presentation will impact the forensic science community by showing a link between the use of science and history to build an archeological detective story. This presentation will also review the evidence to support the claim that Archimedes rightfully is the father of many branches of science who deserves the title of greatest scientific genius of all time.

Archimedes reportedly was born in a Greek colony on the island of Sicily, in the year 287 BC and died in 212 BC. While the year of his death was documented by historical events, the evidence to his year of birth, parental lineage, and early childhood are unknown and subject to conjecture. Archimedes is best known for resolving the issue of the adulteration of gold, used in a crown with baser metal, using then available non-destructive technology. And how he accomplished the difficult quandary, is certainly the stuff that legends are made of. The role of this scientific genius in the planning of the defenses around Syracuse, and his participation during the Second Punic war is less

known. There are only speculations about the manner of his death during the rout and rape of Syracuse and the carnage that followed.

The reason for these large gaps in our knowledge and appreciation for the works of Archimedes stems from the lack of documentation about his publications. Almost nothing of his original writings or publications has survived the vagaries of nature, time, and war. Archimedes wrote about his research and sent it out to associates, none of which has survived. Some of his material was translated and preserved in other languages and these have provided limited insights in to the mind and work of this great man of science. There was some additional information in commentaries by scholars who wrote about him many years after he died. Somewhere along the line, facts blurred and truth was supplanted with fiction and evolved into legends.

The first comprehensive compilation of the mathematical writings of Archimedes was made in 530 AD, and commentaries of his works made in the $6^{th}$ century opened his work to scientists during the Renaissance period. The discovery in 1906 of the previously unknown works *The Archimedes Palimpsest,* was the first modern day look into how this great man of science obtained the amazing results of mathematical calculation far ahead of his time. This manuscript on parchment was first written, then scraped over and rewritten (as was the literary fashion of the time). Professor Johan Ludvig Heiberg realized the 174-page parchment with $13^{th}$ century prayers overlay a $10^{th}$ century writing about the previously unknown works of Archimedes. After spending hundreds of years in a monastery library, the first modern translation of the writings, with numerous limitations, was made approximately a hundred years ago.

The limitation of Heiberg's work was that it interpreted only the words of the text without the diagrams. The document disappeared again and resurfaced in 1998, and was sold to an anonymous buyer. As described in the book *The Archimedes Codex*, the Palimpsest has now been reinterpreted using the more modern scientific techniques of image analysis as well as paleographical (script deciphering) and philological (text–analyzing) skills of modern day scholars. The Codex itself is now stored at a museum in Baltimore, Maryland and is an amazing example of bringing to life the ancient texts.

This presentation will review the available information, confirmatory tests to prove its veracity, and attempts to separate the truth from the legends. Since no good forensic tale is complete without a death and a mystery, the circumstances of the death of Archimedes and the speculations about the manner of his dying will be briefly outlined.

**Archimedes, Genius, Scientist**

# LW4 Death of a Vampire?: Case of Exhumation and Mutilation of a Corpse in Rural Romania

*William C. Rodriguez III, PhD\*, Armed Forces Medical Examiner, 1413 Research Boulevard, Building 102, Rockville, MD 20850*

After attending this presentation, attendees will have a better understanding on how the misinterpretation of decompositional artifacts has contributed to beliefs, superstitions, and the myth about the existence of vampires. Attendees will be shown a video clip of the actual forensic grave side examination of a reported "vampire" who was put to his final death by family members.

This presentation will impact the forensic community by educating the attendees on the various folklore associated with the legend of vampires. Secondly, scientific explanations involving postmortem changes will be reviewed and utilized to explain misconceptions about changes in the human body after death which lead individuals to place their belief in vampire myth and legend.

Even before the famous classic tale of horror "Dracula", written by Bram Stoker in 1897, the belief of vampires can be traced back as far as the fifteenth century to various parts of Europe. Of all the various countries and regions steeped in the belief of vampires and the undead, none run so deep as in the country of Romania. The belief in vampires is rooted in many cultural beliefs regarding the afterlife such as the acknowledgement of Satan and his monstrous minions. Another important aspect of the belief in vampires is based on the misunderstanding of the changes that occur to the body as the result of the decompositional process. Misunderstood changes include the postmortem purging of bloody fluids from mouth and nose which were thought to be evidence of recent feeding, and the appearance that the hair and nails continue to grow after death. Other examples of misconception include the presence of guttural sounds from the deceased as the result of expelled postmortem gases, and the postmortem pink and reddish discolorization of the skin gave the appearance that a corpse had returned to life.

A prime example of the deep seated cultural belief in vampires in parts of rural Romania is demonstrated in a recent case which involves the exhumation and mutilation of a corpse. In December of 2003 a 76 year-old retired school teacher in the rural Romanian village of Marotinul de Sus died. At his death, the elderly male was placed in a simple wooden coffin, which was then buried in a shallow grave located below a make-shift stone vault. Later in time various relatives of the deceased begin to fall ill and claimed to have had dreams in which the deceased had risen from the dead as a vampire to drink their blood. As a result of the unexplained illnesses and terrifying dreams, several family members made the decision to follow the ancient cultural tradition, and destroy their now believed undead family member.

In July of 2005 six family members traveled to the cemetery under darkness and exhumed the body of their deceased relative. Waiting to the stroke of midnight, a member of the group drove a pitchfork into the chest of the corpse, then opened the chest cavity with a large knife, and removed the heart. The corpse was then repeatedly stabbed in various locations with wooden stakes and garlic sprinkled over the body. The group departed the cemetery with the heart impaled on the pitchfork and proceeded to a near by crossroads. At the crossroads, the family members burned the heart, then mixed the ashes with peppermint schnapps, and drank the concoction. As a result of their actions, they no longer felt ill, and their terrible dreams of their vampire relative were not repeated.

Later in time, word of this macabre ritualistic act made its way to the daughter of the deceased and local authorities. A second exhumation of the corpse was ordered by authorities investigating the horrific act, in which a grave side forensic examination was conducted by a forensic pathology team. The grave side examination by the forensic pathology team corroborated the story of the mutilation, including the removal of the heart. A video clip of the actual grave site examination will be presented.

As a result of the seemingly indignant and horrid act, the six family members who had participated in the mutilation of the corpse were arrested and sentenced to six months in jail. The arrest of the family members greatly angered local villagers who indicated that this was a practice that had been conducted by locals for many centuries. Many villagers praised the action carried out by the six, noting that it was a great thing to take out his heart as the people were in danger. Other villagers confessed to have taken the hearts from the dead many times before, and to have drunk a solution containing the ashes of the heart. In their own defense, the leader of the six family members pleaded innocent to having done nothing wrong. The leader informed the police that when they exhumed the corpse he had blood surrounding his mouth, and that he moaned when they stabbed him with the pitchfork. Pleading with authorities, the head family member stated that if he hadn't conducted the ritual, that his son, wife, and daughter-in-law would have died.

**Decomposition, Postmortem Mutilation, Ritual**

## LW5 The Great White Dope: Was Jim Jeffries Drugged Before His Heavyweight Title Fight With Jack Johnson?

*James A. Filkins, PhD\*, Office of the Medical Examiner, 2121 West Harrison Street, Chicago, IL 60612*

After attending this presentation, attendees will have a better understanding of the various explanations for vague symptoms of "doping."

This presentation will impact the forensic community by informing the forensic community of a little known, but interesting historical case of suspected poisoning.

In 2007 George Foreman announced that he lost his 1974 heavyweight title fight with Muhammad Ali – the "Rumble in the Jungle" – because he had been drugged. Foreman's revelation was not the first time that a former heavyweight champion claimed that he lost his crown because he had been "doped."

On July 4, 1910, heavyweight champion Jack Johnson fought the "Great White Hope," former heavyweight champion Jim Jeffries, in Reno, Nevada. Although the "tale of the tape" favored Jeffries, age and diminishing skills did not.

Jeffries had been born in 1875. He won the heavyweight title on June 9, 1899 by knocking out Bob Fitzsimmons in the eleventh round at Coney Island, New York. Jeffries stood six feet two inches and weighed 206 pounds when he won the title. He had a reach of 76 ½ inches. Jeffries retired in 1904 with a record of seventeen wins, two draws, and no losses. Fourteen of his victories came by knockout. After his retirement, Jeffries did not fight again until he began training for his comeback against Johnson. In the intervening years, his weight ballooned to almost three hundred pounds. Jeffries died in 1953 of natural causes.

Jack Johnson was the younger man by three years. He took the title from Tommy Burns on December 26, 1908 in Sydney, Australia. Johnson won a unanimous decision when police stopped the bout after fourteen rounds. Johnson was six feet one and-a-half inches and weighted 192 pounds when he beat Burns. His reach was 74 inches. When Johnson finally retired in 1938 at the age of 60, his record stood at eighty-two wins, fourteen losses, and ten draws. He scored fifty-one knockouts. Johnson died in 1946 in an automobile accident. Significantly, in the six years between Jeffries's retirement and Johnson's match with the former champion, Johnson's boxing skills and reflexes had been sharpened by thirty-seven fights, of which he lost only two.

On the day of the fight, Jeffries described himself as having become "weaker and more sluggish" every day for a week, although he experienced no pain. In his own words, he was "dull, listless and numb," and suffered from "dysentery." On the morning of the fight, Jeffries overslept. His handlers had difficulty waking him and when they finally did, noted that his body was as "cold as ice." When Jeffries tried to warm up, first by walking and then running, he stumbled so much that some thought he was intoxicated. By his own admission, Jeffries had nothing to drink that day — not even water — except a glass of champagne. As the fight was about to begin, Jeffries became chilled again, even though the temperature in the ring approached 100 degrees Fahrenheit. Not surprisingly, Johnson easily dominated Jeffries and retained his title with a fifteenth round technical knockout.

A few days later Jeffries's brother and others claimed that the former champion had been "doped" before the fight. Jeffries initially denied the rumors, but reversed himself with the publication of his authorized biography in 1929. He claimed that his physician, a leading Los Angeles internist, confirmed that he been drugged and that he had continued to suffer from the drug's ill effects until 1927. Although Jeffries described his symptoms, he never identified his doctor or the drug, and only vaguely hinted at who may have given the mysterious substance to him.

Was Jeffries's claim simply a proud, old champion's attempt to rationalize his only defeat or was he really "doped?" If so, what might the drug have been, who gave it to him, and how might it have been administered?

**Boxing, Poisoning, Doping**

## LW6 Slavery in Lincoln's Illinois: John Hart Crenshaw and the Old Slave House

*Darlene Shelton, PhD\*, Emancipation Institute, Inc., 3 Taliar Ridge Road, Guilford, CT 06437*

After attending this presentation, attendees will become familiar with an elaborate interstate criminal enterprise for kidnapping, enslaving, trafficking, and breeding free African-Americans that operated in Southern Illinois and neighboring states from 1840 to 1860.

This presentation will impact the forensic community and those in law enforcement, human behavior, engineering, and jurisprudence who are concerned with missing persons, kidnapping, human trafficking, slavery, and torture.

From 1840 to 1860, while Abraham Lincoln was establishing his reputation as a talented orator, attorney, and politician in Southern Illinois, another individual, John Hart Crenshaw was creating an interstate crime organization fueled by greed, cruelty, corrupt politics, and callous disregard for the law. Based in the town of Equality, Illinois, the criminal operation preyed on freedmen and their families drawn to southern Illinois by fertile farmland and large communities founded by former slaves – Locust Grove in Williamson County and Miller's Grove in Pope County. Crenshaw's Gallatin County adjoined these settlements and his crime ring was dedicated to kidnapping, enslaving, and breeding his African American neighbors, including children as young as seven years old.

John Hart Crenshaw employed an elaborate criminal network of family and friends, using hideouts and holding stations in western Kentucky, southern Illinois, and an area of Missouri that stretched from St. Louis to the southern tip of the state's boot heel. Crenshaw's occasional brushes with the law had little consequence. He successfully laundered his criminal profits through legitimate businesses while his occasional brushes with the law were inconsequential. Crenshaw and his wife, the former Sina Elizabeth Taylor, enjoyed a prominent place in local society. Crenshaw's wealth and land grew exponentially as he exploited the slave labor of his kidnapped victims to mine salt from the natural mineral-rich springs of present day Saline County.

As Crenshaw's interests grew, he sought to expand his political influence. He was one of the few Illinois Democrats to support a system of internal improvements, and probably conferred with one of the system's staunchest proponents, the Whig minority leader, Illinois Representative Abraham Lincoln. Local tradition says that Crenshaw held a dance in Lincoln's honor and hosted him overnight in his home, the Hickory Hill Plantation House. Built in 1838, the Hickory Hill Plantation House, known today as "The Old Slave House," still stands, the only remaining structure of its type. The house was specifically designed to hold kidnapped African-American men, women, and children. The captives were held on the third floor, which includes a series of small wooden cells, a whipping post and markings on the floors and walls from chains and leg irons which restrained the victims.

Crenshaw's kidnapping and slavery operation was one of the longest operating slavery rings in U.S. history. His cruelty and sexual depravity became legendary and still persist in the oral tradition of Southern Illinois.

**Kidnapping, Slavery, Racketeering**

## LW7 Recent Developments in the Ongoing Saga of the Houston Mass Murders

*Jennifer C. Love, PhD, Harris County, Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054-2098; Jason M. Wiersema, PhD, and Ruth A. Mathis, Harris County Medical Examiner's Office, Anthropology Division, 1885 Old Spanish Trail, Houston, TX 77054; and Luis A. Sanchez, MD and Sharon M. Derrick, PhD\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054-2098*

After attending this presentation, attendees will learn about the ongoing efforts and recent breakthroughs by the Harris County Medical Examiner's Office (HCME) Forensic Anthropology Division (FAD) in obtaining identities for the three remaining unidentified decedents of the Houston Mass Murders of 1973 which will be described in detail. Attendees of this presentation will receive a brief background overview of the first large-scale serial murder case in the United States, a four-year binge of violence that resulted in the deaths of 27 adolescent males. Early efforts to identify the decedents, the disposition of 24 cases, and a description of the current status of the unidentified cases will be discussed.

This presentation will impact the forensic community by detailing successful standardized methods used by a formally organized medical examiner identification unit to obtain identities in cold cases.

August 8-9, 2008 marked the 35th anniversary of the discovery of 27 adolescent males between the ages of 13-21 who had been killed and then buried in three locations in the vicinity of Houston, Texas over a four-year period. The perpetrators, Dean Corll (33), Elmer Wayne Henley (17), and David Brooks (18) collected the majority of the teens from the Heights neighborhood in the heart of Houston. The cause of death was found to be either strangulation or gunshot wound(s), and at least one decedent was subjected to genital mutilation. The salacious aspects of the murders, the youth of the victims, and the potential for complaints against local law enforcement's handling of missing persons cases engendered a frenzy of local and national media attention. The local media dubbed the cases "the Houston Mass Murders." The term "serial murder" had not yet been invented.

At the inception of the FAD in 2006, the cases of the three remaining unidentified victims of the serial murders were assigned for follow-up and they received the standard review that is FAD protocol for cold cases. A skeletal analysis was performed by a staff anthropologist, a biological profile was obtained, the permanent case records and identity tracking sheets were reviewed, and bone samples were submitted to the University of North Texas Center for Human Identification for DNA extraction and analysis. Additionally, one decedent received three facial approximations and two decedents each received two facial approximations. Due to the efforts to place case information on missing persons websites and renewed media interest as the anniversary of the crimes approached, the facial approximations have been widely disseminated.

In the second phase of attempting to solve the identity mystery, one of the more reclusive perpetrators, David Brooks, was interviewed at the correctional facility where he is incarcerated. He was willing to provide some additional information after viewing the facial approximations. Internet search engines were used to locate families of possible victims names gleaned from the old HCME records and police reports. This was made more difficult because, after approximately 35 years, many relatives had moved several times and the older family members are deceased. Reports of successful extraction of mtDNA and autosomal DNA from the remains of all three decedents provided encouragement to press on. As of this writing, close family members of four missing teen boys from the time period, three who were mentioned as possible victims in the archived records and one whose niece saw the news reports, have submitted DNA buccal swab samples. Results of comparisons with the

decedents are pending. A number of peers of teen boys who went missing from Houston in the early 1970's have, as a result of the news reports, called with information. These calls generated a new list of names to investigate further.

Positive identification of these teens will allow families to provide for final disposition of the remains and to have a long-awaited answer to their questions regarding the disappearance of their loved one. This would be a very rewarding conclusion to the saga.

**Unidentified, Henley, Mass Murders**

## LW8   Closing In On a Cold Case: Boulder Jane Doe

*Silvia Pettem, BA\*, Book Lode, LLC, 439 Ranch Road, Ward, CO 80481; Walter H. Birkby, PhD, Forensic Science Center, 2825 East District Street, Tucson, AZ 85714; Frederick A. Bornhofen, BS, 1 Sunmere Lane, Elverson, PA 19520; Todd W. Fenton, PhD, Michigan State University, 354 Baker Hall, Department of Anthropology, East Lansing, MI 48824; Richard C. Froede, MD, 3930 North Placita de la Escarpa, Tucson, AZ 85750; and Phil West and Steve Ainsworth, Boulder County Sheriff's Office, 1777 6th Street, Boulder, CO 80302*

After attending this presentation, attendees will learn how law enforcement officials, forensic specialists, and a historian combined their expertise to uncover half-century-old data on a very cold homicide.

This presentation will impact the forensic community by showing how old-fashioned "gum-shoe" research was mixed with modern technology to bring a long unsolved cold case closer to closure.

In April 1954, two University of Colorado college students stumbled upon the naked body of a young woman in Boulder Canyon, west of Boulder, Colorado. The only clues to her identity were two bobby pins in her long wavy hair. After much publicity in the Boulder-Denver area, members of the community raised the funds to bury the unidentified murder victim in Boulder's Columbia Cemetery. Her gravestone read, "Jane Doe, April 1954, age about 20 years."

Fifty years later at the urging of the historian of Boulder County, Sheriff Joe Pelle reopened the Jane Doe homicide case, pending funds. Again, the community sent in their checks and the Vidocq Society stepped forward to aid in the investigation.

The original case file (along with others of the era) was missing, so the historian reconstructed a new case file from old newspaper articles. The newspapers' descriptions and photos of the crime scene led to its exact location, as determined by four large and distinct rocks which hadn't moved in half a century.

Vidocq Society members assisted the Sheriff's office in exhuming the victim's remains in June 2004. The casket had disintegrated underground, so the exhumation resembled an archeological dig that lasted two days. DNA was obtained from one of Jane Doe's teeth, and Dr. Walter Birkby, a forensic anthropologist, reassembled her skull from more than 100 pieces. Forensic artist Frank Bender sculpted the victim's facial reconstruction and presented it to the public in 2005. In 2006, the case was shown to a national television audience on "America's Most Wanted." There were no credible leads.

Meanwhile, two women were ruled out by DNA comparison. The historian then supervised a team of researchers who whittled down their list (compiled mostly from newspaper accounts) of missing young women. Katharine E. Farrand Dyer, an elevator operator who had disappeared from Denver thirteen days before the body was found, rose to the top of the likely candidates. At the same time, then Denver resident Harvey Glatman was, and still is, considered to have been her murderer.

To date, no maternal blood relatives of Dyer's have been found in order to make a positive DNA identification. The historian, however, located the young woman's photograph which Dr. Todd Fenton superimposed on a cast of Jane Doe's reassembled skull. The match was so close that Dyer could not be excluded as Jane Doe.

The Boulder County Sheriff's Office has reburied Jane Doe in her former grave in Boulder's Columbia Cemetery.

**Cold-Case Homicide, Historical Research, Superimposition**

## LW9   Bat Masterson's Denver Adventures

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After attending this presentation, attendees will have an enhanced and better understanding of Colorado and Western history from the not too distant past. Bat Masterson's activities covered both his service as a lawman and U.S. Marshall, as well as a person who picked and chose which laws to heed.

This presentation will impact the forensic science community by giving them reasons to contemplate contemporary law enforcement and how it has changed and not changed from the antics of Bat Masterson until now.

Bat was born in Canada in 1853 and died in New York City in 1921. His first gunfight occurred in Texas when he was 23. He came to Colorado in that same year where he served as a sheriff for three years. He roamed from state to state throughout Kansas, Colorado, and Arizona but had settled and perhaps married in Colorado in the 1880s. Here he set up a theater and gambling establishment, the Palace, opened a local athletic club, and wrote a weekly sports column for a Denver paper. Masterson left Colorado in something of a hurry for New York in 1902, but memories of him and monuments to him remain here. In New York he is memorialized as Sky Masterson in the Broadway hit, Guys and Dolls.

**Frontier Lawman, Gambler, Newspaper Reporter**

## LW10   Alfred G. Packer Victims' Exhumation Project Revisited

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After attending this presentation, attendees will be reminded of the original results of the Alfred G. Packer Victims' Exhumation Project as determined by the forensic investigators who performed the exhumation of the mass grave and the subsequent skeletal analyses of the five ill-fated prospectors.

This presentation will impact the forensic science community by addressing the claims made by subsequent investigators that run contrary to the results as determined by the original research team.

Nearly twenty years have passed since Scientific Sleuthing Incorporated (SSI) sponsored the exhumation of the five prospectors who are believed to have been murdered and cannibalized during the brutal winter of 1856 by the notorious Alfred G. Packer. Well-known to many as the Colorado Cannibal, Packer was the lone survivor of a failed expedition to find gold in southwestern Colorado's San Juan Mountains. Packer was convicted in a court of law for the murders of the five prospectors after their skeletal remains were found several months after their deaths by a travelling magazine sketch artist.

The site of this grisly discovery was a picturesque plateau above the beautiful Lake San Cristobal near present day Lake City, Colorado. As history recorded the event, the remains of the five men were interred in

a common grave, marked with five wooden crosses near the site of their recovery.  It was at this grave site during the pleasant summer of 1989 that a team of forensic investigators sponsored by SSI attempted to utilize modern medicolegal techniques to address three century-old questions:  1) Were the five prospectors actually buried in the alleged grave site?;  2) Was there evidence that they had been murdered?;  and 3)  Was there evidence that their bodies had been cannibalized?

The first of these questions was rather quickly answered when the skeletons of five adult men were exposed within their resting place.  Not long after the skeletons were freed of the adherent soil, and still *in situ*, evidence of blunt-force trauma was seen on all of the skulls.  Addressing the final question would require laboratory analyses and thus the five skeletons were removed from the common grave for a 600 mile trip to Tucson, Arizona for analyses and documentation.

Lab analyses at the University of Arizona confirmed that each of the five men displayed evidence of being defleshed of varying amounts of their muscle tissue.  Additional laboratory analyses revealed that the ages of the five men were consistent with that of the prospectors and that one of them had suffered an injury during his life that did not heal properly.  This latter finding would prove instrumental in effecting circumstantial identifications for the five prospectors.

Evidence of postmortem scavenging of the skeletal remains was manifest and further analyses revealed the extent of the blunt-force trauma to the skulls and the sharp-force cuts that marked the sites of defleshing.  No evidence of perimortem gunshot trauma was present to any of the skeletons.  This finding takes on added importance because of two factors:  1) Packer contended that he shot one of the prospectors in an act of self-defense; and 2) Recent investigators have postulated that one of the skeletons does exhibit evidence of a gunshot wound.  Not only is this contention disputed, but the prospector that reputedly has the gunshot wound is not the same one that Packer claimed to have shot.  The findings from the original forensic investigation will be reviewed in light of some of these recent claims that run contrary to our initial results.

**Cannibalism, Postmortem Scavenging, Victim Identification**

# PRESENTING AUTHOR INDEX

Decker, Summer J.-D40, H31, H59
DeGreeff, Lauryn E.-A49
Deisch, Jeremy-G5
DeLonga, David M.-G110
Derrick, Sharon M.-H112, LW7
Desiderio, Vincent J.-A68, S3
Desranleau, Sylvain-F6
DeWitt, Grant W.-C12
Di Vella, Giancarlo-I22
Diaczuk, Peter J.-A109, W4
Dicke, Clarissa R.-H80
Diegoli, Toni Marie-A21
Diekema, David A.-C19
Dionne, Charles A.-H82
Dirnhofer, Richard-W14
Dixon, Alan B.-A173
Doctorman, Christopher-K8
Dooley, Melinda K.-G75
Dos Santos, Alex A. Da Luz-F7
Dowdy, Liotta N.-H77
Downs, J.C. Upshaw-BS3, G104
Doyle, Sara Nunez-G56, G106
Drew, Robert W.-W17
Driscoll, Kathryn R.D.-H78
Driscoll, Rachel M.-A35
Driscoll, Robert-A93
Druid, Henrik-G109
Dudley, Mary H.-G48

## E

Eberhardt, Troy J.-W7
Eckenrode, Brian Allen-A44, D11
Eckert-Lumsdon, Tiffany L.-K41
Edson, Suni-A130, H12
Edwards, Carl N.-E1
Ehrhardt, Christopher-A135
Einseln, Anja-W16
Elian, Albert A.-K2
Elkins, Kelly M.-A19, A102
Elrick, Douglas G.-B3
Emanovsky, Paul D.-H76
Escuadro, Marie Elaine Batto-A127

## F

Fahrnow, Valerie K.-E8
Fais, Paolo-G14
Falsetti, Anthony B.-LW1
Faria, Mafalda S.-A51
Farrugia, Audrey-G117
Faugno, Diana-D9
Felix, Jeremy-A41
Fenton, Todd W.-H38
Ferraro, Chantal-G49
Feulner, Mark-D45
Fierro, Marcella F.-S1
Filippi, John E.-G48
Filkins, James A.-LW5
Fisher, Barry A.J.-A79
Fixott, Richard H.-F17
Fleischman, Julie M.-H40
Flores, Micah-G34

Fontana, Donna A.-H55
Ford, Jonathan M.-H31
Fordham, Judith-E2, E6, E15
Forney, Peggy J.-C13
Fottrell, James-S2
Fowler, David R.-W14
Franicevic, Branka-H6
Fujikawa, Amanda-G66
Fukushima, Hirofumi-A15
Fulginiti, Laura C.-H90
Funes-Huacca, Maribel E.-A18
Funk, Christine-W15
Furbish, Christopher J.-C6
Furgeson, Thomas A.-H52
Furton, Kenneth G.-A118

## G

Gagliano-Candela, Roberto-K23, K29
Gallo, Jenny-A32
Gantz, Donald T.-J2
Garcia-Rivera, Enid J.-D35
Gardner, Elizabeth A.-A166
Gascon, Santiago-A53
Gauthier, Kellie M.-A23
Geberth, Vernon J.-BS2, S3, W2
Gefrides, Lisa A.-A132
Geraci, Alberto L.-C2
Geradts, Zeno J.-B19, B20, W19
Geraut, Annie-G81
Gerns, John E.-S3
Gerretsen, Reza R.R.-D14
Gershaw, Cassandra-A25
Gestring, Brian J.-A69, B24
Getz, John M.-W14
Gialamas, Dean M.-W18
Gibelli, Daniele-G6, H69
Gibson, Laura E.-H85
Giles, Robert C.-W11
Gilliland, M.G.F.-G40
Gilliland, Rhesa G.-S3
Glock, Lindsey-F26
Gobelet, Barrett-H36
Godde, Kanya-H24
Gofton, Jeffery J.-G63
Golden, Teresa D.-A117
Goldschmidt, Ariel-G52
Gonzalez, Brittney C.-A42
Goodpaster, John V.-A72, A152
Gordon, Ann M.-K34
Gormley, William T.-G60
Grammer, Scott R.-A61
Graves, Matthew E.-B27
Gray, Teresa R.-K3
Greaux, Jennifer L.-K20
Green, Jody L.-G45
Green, Robert N.-E7
Green, William-BS6
Grillo, Massimo-D30, G115
Gromb, Sophie-E17
Guerra, Patricia-A148
Gunn, Joshua A.-W13
Gunther, Wendy M.-G61, G63
Guzy, Gerald-F27

## H

Hackett, Jeffery-K1, W8
Hackman, Lucina-H65
Haden-Pinneri, Kathryn H.-G116, H58
Hagan, Kristin A.-A100
Hagen, Margaret A.-E1
Hall, David W.-G34
Hamilton, Michelle D.-H97
Hammond, Derek L.-J12
Hanson, Ian-W24
Hanzlick, Randy L.-S1
Harcke, H. Theodore-W14
Harding, Brett E.-D6
Harmon, Rockne P.-E7
Harrell, Caleb A.-K24
Harris, Heather L.-E16
Harris, Kyla E.-A98
Hart, Gina O.-H51
Hart, Walter-S2
Haupt, Trista M.-K14
Heartsill, Chris-K32
Hefner, Joseph T.-H106
Henderson, Julie A.-H110
Heng, Lei-A124
Herrick, Christen E.-H42
Herschaft, Edward E.-F22
Hibbard, Ryan-A142
Hill, Vincent E.-G50
Hill, Whitney-S3
Hilts, Mark-W17
Hinton, William L.-B28
Hinz, Andrew-A78
Hirtzel, Angela-A36
Ho, Jeffrey D.-G121
Hodgins, Gregory W.-G101, H4
Hoffman, Thomas R.-I9
Hogan, Stephen P.-E3
Hogue, Jennifer M.-K17
Holmgren, Janne A.-E2
Hoogeboom, Bart-B21, W19
Hoogewerff, Jurian A.-A106
Horvath, Frank-D36
Houck, Max M.-A2, A66, S2, W18
Houlgrave, Stephanie M.-J18
Howald, Blake S.-E11
Huang, Tsun-Ying-A20
Huard, Aimee E.-H37
Huggins, Lesley Ann M.-A109
Hughes, Cris E.-H21
Hulsey, Brannon I.-H9
Hurst, Carolyn V.-H108
Hutchings, Kim E-D7
Hyzer, James B.-C11

## I

Illescas, María J.-G26
Inman, Keith E.-A67
Isenschmid, Daniel S.-K58
Ivory, Matthew A.-C5, C10

## J

Jackson, Rick-W3
Janis, Gregory-W8
Jarzombek, Jacqueline M.-A52
Johnson, Amanda-H95
Johnson, Christopher-A82
Johnson, Donald J.-A128
Johnston, Cheryl A.-H99
Joiner, Patricia J.-A115
Jones, A.W.-K40
Jones, Gabrielle-A108
Jorden, Michelle A.-G103
Jouett, Jamie L.-D10, K15
Jousset, Nathalie-G33
Juarez, Chelsey-H22, H116
Jurgens-Toepke, Pamela-F8

## K

Kanchan, Tanuj-D3, H70
Karch, Steven B.-G58
Karwacki, Lisa A.-A143
Kaufman, Stacie R.-A189
Kavlick, Mark F.-A129
Kelley-Primozic, Kirsten T.-A27
Kelly, Charles M.-A122
Kemp, Bobbie J.-H10
Kendell, Ashley E.-H81
Kennedy, Ronald C.-W9
Kester-Florin, Sara J.-K9
Kimmerle, Erin H.-H109
King, Damon-S2
Kish, Paul E.-A9
Klales, Alexandra R.-H23
Klotzbach, Heike-G102
Kocaelli, Humeyra-F29
Komar, Debra-H67
Koons, Robert D.-A156
Koskinen, Mikko T.-A88, A139
Kriigel, Carl-W6
Kroll, Mark-C18
Kroman, Anne M.-H48
Kronstrand, Robert-K48
Krouse, Marc A.-D21, G11
Kubic, Thomas-A65
Kubicz, Elzbieta J.-A40
Kugelberg, Fredrik C.-K49
Kusano, Maiko-A37
Kutyla, Alicja K.-H47

## L

La Harpe, Romano-D19, W2
LaBerge, Greggory S.-E7
Lachowicz, Marrah E.-G15
Lai, Hanh-A149
Landers, James P.-W11
Langman, Loralie J.-K35
Lann, Meredith A.-G16, G22
LaPorte, Gerald M.-J17, W7
Ledesma, Yvonne-D2

Ledford, Jennifer A.-H36
Lee, Henry C.-ES1
Lee, Steven B.-A15
Lee, U-Young-H25
Leestma, Jan E.-G47, W9
Lemos, Nikolas P.-K38
Lenda, Kimberly-K27
Lennard Richard, Mara-A99
Lentini, John J.-A140, W18
Levin, Alan S.-W9
Lewin, John-W3
Lewis, Don L.-B16
Lewis, Jane A.-S3
Lewis, Russell J.-K39
Li, Ling-G35
Lichty, Jessica E.-A11
Liddicoat, Laura J.-K37
Limoges, Jennifer F.-K33
Lin, Wen-Ling-K25
Liptai, Laura L.-S3
Lipton, Barry E.-S3
Listi, Ginesse A.-H73
Liszewski, Elisa-A75
Lobo, Stany W.-H17
Lockwood, Randall-G34
Long, Benjamin-B6
Looman, Karen B.-G38
López, Magdalena-I5
Lorente, Jose A.-A170
Lorin de la Grandmaison, Geoffroy-G111
Lounsbury, Jenny A.-A16
Love, Jennifer C.-H58
Lovelace, Rachel-A47
Lucas, William J.-D20
Lurie, Ira S.-W13
Lyle, James R.-B8

## M

MacGregor, Donna M.-H14, H87
Macias, Michael S.-A165
Majidi, Vahid-E13
Malone, Christina A.-H111
Manishen, Jeffrey R.-G46
Mann, Gina-A19
Manzolillo, Patricia-W6
Marcia, Tim-W3
Mario, John R.-W18
Marks, Murray K.-H91
Marr, Kenneth-B28, S2
Marschner, Julie M.-A23
Marshall, Amanda J.-G27
Martell, Daniel A.-E1
Martin, Audrey N.-A188
Martin, Thomas L.-E3
Martinez, Richard-I1
Martinez, Vanessa L.-A157
Mashali, Amal A.-D15, D42
Mason, Donald S.-W13
Matheson, Gregory B.-A54
Mauillon, Damien-D32
Maujean, Géraldine-D5
May, Shannon E.-H74

Mayntz-Press, Kathleen A.-A83
McBeth, Molly-A85
McCary, Alexandra-A134
McCauley, Heather A.-A164
McClellan, Ashley L.-A59
McCord, Bruce R.-W13
McCoy, Mark R.-B23
McFarland, Mark A.-B18
Mclendon, Marna-E6
McManus, Sarah E.-H18
Meade, Jennie-LW2
Meehan, Audrey L.-H61
Mehltretter, Andria H.-A185
Meininger, Marissa R.D.-A126
Melbye, Jerry-H8
Melson, Kenneth E.-ES2
Meneses, Breeanna N.-A12
Menezes, Ritesh G.-G31
Mercedes, Fernando-G26
Mercer, Stephen B.-E7
Merck, Melinda-G34
Metcalf, Roger D.-F16
Michaud, Katarzyna-G82
Micheals, Anastasia D.-C3
Middleberg, Robert A.-W5
Miles, Harry L.-E10
Miller, John J.-J2
Miller, Raymond G.-F12
Millette, James R.-C14
Mishalanie, Elizabeth A.-A178, W20
Moenssens, Andre A.-E1
Mogle, Mark-W16
Mohammed, Linton-J11
Mohr, Rachel M.-G68
Mohrhaus, Angela S.-A116
Mokrzycki, Gregg Mitchell-J13
Molina, D. Kimberley-G16
Moloughney, Kerri Lynn-D34, J9
Moody, Christopher M.-A114
Moorehead, Wayne-W4
Morgan, Stephen L.-A10
Moriarty, Jane C.-E1
Morrissey, Mitch-E7
Morton, Robert J.-W17
Moser, Lauren E.-G42
Moss, Kathryn E.-H43
Moudouni, Mounir-A55
Mozayani, Ashraf-W16
Mulligan, Christina M.-A57
Murphey, Carey R.-B2
Musheno, Thomas-W6

## N

Nair, Mohan-I10
Nance, Gerald N.-W3
Nase, Philip S.-A62
Nashelsky, Marcus B.-H33
Nawrocki, Heidi-S3
Nawrocki, Stephen P.-H92
Neal, Samantha H.-W1
Neece, Timmy L.-G90
Neri, Margherita-G80

Swiatkowski, Sean A.-G37
Swienton, Anjali R.-W15
Symes, Steven A.-BS7
Synstelien, Jennifer A.-H101

## T

Tambuscio, Silvia-G78
Tan, Eugene-A176, A177
Tape, Christopher-G88
Taylor, Allison G.-A80
Taylor, Catherine N.-A95
Telepchak, Michael J.-W8
Tetreault, Michelle E.-A186
Tettey, Justice Nii Addy-D28, W16
Thali, Michael-S2, W14
Thevissen, Patrick W.-F15, F25, F37
Thogmartin, Jon R.-G51
Thomas, Jennifer L.-A96, A168
Thompson, Christopher R.-I16
Thompson, Elizabeth-G30
Thomsen, Asser H.-G20
Thrane, Craig-W6
Thurmond, Sylvia-A26
Tidball-Binz, Morris-G54
Timanova, Anna-A103
Tirado, Carmen A.-G26
To, Denise-H26
Tournel, Gilles-G120
Tramell, Michael S.-I20
Tregar, Kristen L.-D41
Trejos, Tatiana-A73
Troccoli, Giuseppe-I11
Tuceryan, Mihran-F14
Tucker, Steve-W23
Tumu, Phani M.-I17, I18
Turner, Dee A.-A141
Turnquest, Britt E.-K22
Tytell, Peter V.-W18

## U

Ubelaker, Douglas H.-S3
Uhl, Natalie-H88
Ulmer, Pamela S.-G43
Umback, Noelle J.-A8

## V

Valtier, Sandra-K4
van der Meer, Dirk-F23
van der Peijl, Gerard J.Q.-A110
Van Haeringen, Wim A.-A14, A136
Van Laerhoven, Sherah L.-G97
Vieira, Duarte N.P.-A51, G28, G85
Viel, Guido-G114
Viner, Mark D.-D7
Virgin, George-J14
Vollner, Jennifer M.-H23
Vorder Bruegge, Richard W.-S2, W6, W19
Vredenburgh, Alison G.-I14

## W

Wagner, Michael-K36
Walch, Mark A.-J1, J4
Wallace, Margaret M.-A137
Wallace, William A.-W10
Walls, H. Chip-W22
Walter, Richard D.-W3
Walterscheid, Jeffrey P.-K47, K52
Walton, Richard H.-W3
Wang, John Z.-J10
Wang, Yu-Shan-K16
Watson, Phillip L.-G27
Watsula, Daniel-A63
Watts, Gabriel D.-J13
Watts, Vickie-S3
Wecht, Cyril H.-ES1
Weinstock, Robert-E1, I10
Wert, Heather-A144
Wessling, Roland-H13
Wheat, Amber D.-H102
Whitcomb, Carrie M.-B1, S1
White, Douglas R.-B7
Wictum, Elizabeth-A138
Wiersema, Jason M.-H56
Wiley, Bruce R.-F18
Wilkerson, Jessica D.-G92
Williams, Diane K.-D33
Williams, John A.-H86
Williams, Mary R.-A29
Williams, Philip N.-D12
Williams, Ralph E.-D13
Williams, Robert G.-F16
Williams, Shanna E.-H44, H107
Williams, Timothy L.-G53
Willis, Kevin M.-G94

Willuweit, Sascha-A167
Wilson, Justin L.-A71
Wilson, Rebecca J.-H83
Winburn, Allysha P.-H46
Wingate, Chelsy L.-K5
Wirks, Jessica S.-A38
Wonder, Anita K.Y.-E4
Wood, Robert E.-F36
Wortzel, Hal S.-L2
Wright, Diana M.-A107
Wright, Tracey E.-A123
Wu, Ching-Hong-A147
Wyant, Richard T.-A78

## Y

Young, Laura K.-A46
Yu, Jorn Chi Chung-A182

## Z

Zachariah, Nannepaga Y.-K31
Zeliff, David J.-D23
Zerbo, Stefania-G10
Zhang, Chengcui-B12
Zhang, Xiang-G35
Zlotnick, Joel A.-W7
Zucker, Susan G.-E13

Coronary Artery Stent-G88
Corpora Amylacea-G53
Corrosive Substances-H90
Costs-A66, B3
Cotton-A32
Cotton Fiber-A108
Counterfeit Documents-W7
Counterfeit-J20
Coverings-H5
Covert Installation-B16
Cranial Morphology-H24
Cranial Sexing-H24
Cranial Sutures-H48
Cranial Trauma-F9
Craniofacial Growth-H45
Crash Tests-C4
Creative Thinking-A64
Cremation-H96, H114
Crime-D28, J13
Crime Scene-D23, D26, H14
Crime Scene Collection-A174
Crime Scene Investigation-D29, H53
Crime Scene Recovery-H36
Criminal Investigation-F15
Criminal Mindset-D22
Criminalistics-A1, A3, A70, W4
Cross-Examination-L1
Crushing-D30
Cryptographic Hash-B7
CSI-W24
CSI Effect-E2
CT Imaging-G113
Cult-I9
Currency-A41
Custody-D20
Cutaneous Emboli-G3
Cutting Crime-G14

# D

D21S11-A134
Damaged DNA-C19
Dangerousness-I2
DART™-J17, J19
Data Analysis-A169
Data Measurement-B6
Databanking-A93
Database-A26
Database Search-E7
Databasing-A98
*Daubert*-A12, C1, E1, E3, E6, H75
*Daubert* and *Frye*-A76
Death-D6, D19, G61, G63, G86, H41, K24
Death Investigation-D26, I22, K42
Decalcification-A20
Decay Processes-G102

Deception-D36
Decision Making-I20
Decomposition-F26, G29, G73, G94, H1, H3, H5, H93, H98, H99, LW4
Decomposition Facility-H8
Deconstruction-E9, G44
Decontamination-A128
Deconvolution Tools-A168
Decriminalization-E16
Deep Vent-A24
Degenerate Oligonucleotide Primed PCR-A87
Degraded-A15, A91
Degraded DNA-A45, A92
Demineralization-A130
Demographic Analysis-H81
Dental-F23
Dental Age Determination-F3
Dental Age Estimation-F4
Dental Coding-F31
Dental Forms-F33
Dental Health-H20
Dental Identification-F38
Dental Implants-F30
Dental Radiology-F9, F30
Dental Records-F38
Dentistry-F17
Dentition-H96
Dentures-F24
Departure Angle-BS5
Dependent Minors-I20
Depression-I18
Desktop Scanner-J14
Detection of Manipulation-W19
Detector Dog-A118
Developing Nations-D29
DEXA-H83
Dextromethorphan-G59
DFSA-K20
Diamondoids-A144
Diesel Fuel-A143
Dietary Supplements-A164
Differential Extraction-A16
Diffuse Alveolar Damage-G24
Diffuse Reflectance Infrared Spectroscopy-A10
Digital-B8
Digital and Multimedia Sciences-S2
Digital Evidence-B1, B9, B10, B15
Digital File Formats-B10
Digital Forensics-B2, B11, B15, B23, W10
Digital Imaging-W6
Digital Measurement-J10
Digital Photography-A9, B24
Digital Radiography-F38
Diminished Capacity-I10

Diptera-G67, G71
Direct Analysis In Real Time-A71
Disaster Operations-H54
Disaster Victim Identification (DVI)-H65
Discriminant Function Analysis-H23
Discrimination-A107
Dismembering-G14
Disseminated Malaria-G31
DMSA-K12
DNA-A5, A17, A19, A23, A25, A27, A46, A50, A54, A56, A59, A60, A61, A82, A91, A101, A121, A169, A170, D15, E7, G89, G91, W11
DNA Analysis-D14
DNA Collection-A95
DNA Extraction and Purification-A176
DNA Extraction Kit-A15
DNA Extraction-A94, A126, A175
DNA Profiling-A93
DNA Purification-A126
DNA Quantification-A123
DNA Quantitation-A85, A86, A95
DNA Recovery-A90, A189
DNA SampleMatrix®-A120
DNA Screening Method-A80
DNA Storage-A120
DNA Testing-A7
DNA Typing-A53
Document Examination-A114
Documents-J13
Dog-A138
Dogs-H41
Doll Re-Enactment-D2
Domestic Violence-I4
Doping-LW5
Double Swabbing Technique-A58
Driving-K21, K33, K37, K38
Drowning-G32
Drug-W23
Drug Analysis-W11
Drug Database-K53
Drug Death-G60
Drug Induced Self Mutilation-BS2
Drug Interactions-W22
Drug Quantitation-K51
Drug Seizures-A39, K53
Drug Toxicity-G45
Drugs of Abuse-K3
Drugs-K24, K33, K37, K49
Duct Tape-A56
DUID-K36
Dumpsters-G94
Duodenum-G42
DWI-K32
Dynamic Sampling-A148

# E

Peroxide-A151

Peroxides-A30

Personal Identification-F28, F34, H30, H62, H89

Personality Disorder-I7

Personality Disorders-I11

Peru-H66

Petechiae-G12

Petroleum Hydrocarbons-C12

Phencyclidine-K19

Phenethylamines-K22

Phenol Toxicity-G57

Phenolphthalien-A103

Phenotype Informative SNPs-A133

Philosophy of Science-D45

Phogrammetry-B21

Physical Violence-D5

Physiopathology-G108

Pigmented Inks-A72

Pigments-A74

Pipe Bomb-A60, A152

Pit and Fissure Sealants-F27

Pitchfork-G80

Pitfalls-BS3

Planar Solid-Phase Microextraction-A148

Plastic Bag-G102

Plastic Explosives-A149

Pleading Out-F21

PLM-A179, C15

Poisoning-LW5

Police-H65

Police Chase-G63

Police Interviews-D36

Polylactic Acid-A154

Population Genetics-A167

Population Substructure-A53

Populational Admixture-H107

Portable Radiation Emitting Device-F22

Portable Radiation Emitting Devices-F25

Portable XRF-H11

Portal Hypertension-G39

Positive Identification-G48

Post PCR-A48

Postcranial-H110

Posterior Rib Fractures-G116

Postmortem-D18, K18, K47, K58

Postmortem Anogenital Examination-G55

Postmortem Assaults-F24

Postmortem Autopsy-G19

Postmortem Ethanol-K39

Postmortem Examination-G20

Postmortem Interval-D13, D15, G75, G92, G100, H6, H98

Postmortem Interval-G68, H3

Postmortem Mutilation-LW4

Postmortem Redistribution-W22

Postmortem Scavenging-H101, LW10

Postmortem Submersion Interval-H93

Postmortem Toxicology-K17

Post-PCR Purification-A55

Potassium Hydroxide-G107

Power Fantasies-I21

Power of Attorney-I17

PowerPlex®-A46

Praslin-LW2

Precordium-G52

Predatory Grooming-B14

Preeclampsia-Eclampsia-G86

Pre-Existing Disease-G40

Preferential Lysis-A16

Pregnancy-G39

Preparation-F21

Prescription Drugs-A164

Presumptive-A103

Presumptive Blood Tests-A90

Pre-Trial Preparation-W15

Prevention-G49

Principal Component Analysis-A36

Printing-J14

PRNU-B19

Probability-F14

Processes-B1

Professional Responsibility-ES2

Professionalism-W1

Proficiency-Testing-K13

Profiling-A106

Projectiles-G28

Prolapse Rectum-G57

Promising Practices-BS6

Proofreading Enzymes-A24

Propeller Injuries-H35

Propofol-K42

Prosecution-E17

Prospectors-BS4

Prostitution-D16

Psychiatrists-I13

Psychiatry-I3

Psychological-H7

Psychopathy-I10, I16

Puerto Rico-D1

Pulmonary Arteries-G10

Pulmonary Toxicology-G15

Puma-G30

Pupal Cases-G101

Pupation-G69

Puppe's Rule-G114

Purification-A100

Py-GC/MS-A185

Pyrolysis Gas Cheomatography-A72

Pyrolysis Gas Chromatography/Mass Spectrometry-A186

RT-PCR-A100
Rural-F8
Rwanda-A65

# S

Sacral Auricular Surface Aging Method-H47
Safety System Failure-D17
Safety-I14
Saliva-K57
Sample Preparation-A122, K55
Sampling Strategy-A39
San Francisco-K34, K38
SART Research-BS6
Saw Mark Patterns-A77
Scapula-H88
Scavengers-G27
Scavenging-H94
Scene Investigation-H7
Scene Reconstruction-G43
Scene Recovery-H87
Scent Transfer Unit (STU-100)-A155
Scent Transfer Unit-A49
Science-A3, E6
Scientific Evidence-A79
Scientific Identification-H56
Scientific Investigations-A68
Scientific Method-A65
Scientific Reports-W15
Scientist-LW3
Screening-A81, A162
Search-B9
Search Techniques-H14
Seat Belt Loads-C5
Seat Belts-C4
Seat Failure-C7, C8
Seatback Collapse-C5
Secular Trends-H24
Security-A97
Security Features-W7
Seized Drugs-A160
Seizures-G8
Self-Adhesive Stamps-A94, A128
Self-Defense-A8
Self-inflicted Stab Lesions-G80
SEM-A77, A111, G76
SEM-EDS-C15
SEM-EDX-A180
Semen-A101
Semi-Automated CBCT Separation and Segmentation-F4
Semi-Landmarks-H44
Sensitivity and Specificity-A90
Sensory Deprivation Tank-G22
Septicemia-G38
Sequencing-A98, A134

Sequential Unmasking-A67
Serial Killer-D22, I7
Serial Killers-BS4
Serial Killing-I6
Serial Murder-W17
Serial Murder Dynamics-W17
Serial Sexual Assault-I21
Serotonin Metabolites-K39
Session-S3
Sex and Ancestry Estimation-H23
Sex Assessment-H73
Sex Determination-H25, H27, H28, H70
Sex Estimation-H72
Sex Toys-G79
Sexual-G119
Sexual Abuse-D24, D32
Sexual Assault-A8, A187, D9, K49
Sexual Dimorphism-H26, H71
Sexual Fantasies-I21
Sexual Homicide-G55
Shaken Baby Syndrome-G44
Shark Attack-H34
Shark-Inflicted Trauma-H34
Shell Casings-A63
Shooting Distance-C17
Shooting Reconstruction-A65
Short Tandem Repeat-A131
Short Tandem Repeat (STR)-D10
Short Tandem Repeat DNA Typing-A28
SIBS-A184
Sibutramine-A164
Sickle Cell Trait-G51, G56
Signatures-J11
Signs and Symbolism-D43
Simple-F32
Simulation Training-D7
Sixth Amendment-E5
Skeletal Analysis-D14
Skeletal Fractures-H109
Skeletal Traumata-H40
Skeletonized Remains-A130
Skin-F12
Slavery-LW6
Smokeless Powder-A33, A150
SNP-A17
SNPlexTM-A133
Social Autopsy-G49
Social Networks-E13
Socioeconomic Status-H20
Software-A168, B8
Soil-A34, A106, G69
Solid Phase Extraction-K2, K41
Solid Phase Microextraction-A38, A44, A149, A182
Solving a Case-K26
Soot-C14
South India-D3
Southwest Hispanics-H108

Spam Images-B12
Speaker Recognition-D37
Special-S3
SPECT-L2
Spectrometry-J20
Spectrophotometry-J15
SPE-K1, W8
Sperm-A27
Sphenoid Sinus Fluid-G32
Spinal Canal-G117
Splenic Artery Aneurysm-G39
SPME-A42, A165
Spontaneous Coronary Artery Dissection-G9
Spontaneous Ignition-A140
Spyware-B16
Satistics-A39
SSRI Drugs-K6
SSRI's-K56
Stability-A163, K19
Stabilization-A174
Stalking-I13
Standard Operating Proceedures-B2
Standard Tools-D21
Standardization-D41
Standards-H22, W1, W18
STAR*D Study-I18
Statistical Sampling-A166
Statistics-A76, A105, A135, B21, H75, J2
Stature-H20
Storage Drive-B4
STR-A81, A138, A139, G30
STR Analysis-A94, A122, A176, A177
STR Kits-A22
STR Population Studies-A14, A136
STR Typing-A5
Streptobacillis Moniliformis-G18
Stress-G61
Student-G112
Subadult-H71
Subjective Video Quality-B18
Submerged Vehicles-H92
Subsampling-J3
Sudden Cardiac Death-G82, G115
Sudden Death-G1, G8, G9, G11, G31, G53, G119
Sudden Infant Death Syndrome (SIDS)-G36, G43
Sudden Infant Death-G35
Sudden Infant Unexplained Death Investigation-D2
Suffocation-G78
Sugar-G104
Suicide Pact-G118
Suicide-D19, D21, D35, G103, G106, K23, K31
SUID-D4

SUIDI National Academies-D4
Superimposition-H61, LW8
Supply Chain Operations Reference
    Model-D39
Supra-Inion Depressions-H112
Surface Area-H26
Surfactant-A58
Surveillance Camera-C11
Surveillance Video Quality-B18
Survival-G42
Suspect Activity-A188
Suspected Child Abuse-H40
Suture Fusion-H48
Swabbing Mediums-A57
Swabbing-A59
Sweat-A43
SWGDOG-A118
SWGDRUG-A160
SWGIT-W6
Synthetic Opioids (Opana)-G62
Synthetic Standard-A129

## T

Tandem Mass Spectrometry-K8
Taphonomy-BS7, G73, G94, G102, H6,
    H100, H101
Tarsals-H28
TASER®-C18, G17, G121
TATP-A30
Technology-A3, D40, ES1
Teens-H45
Television-G5
Temperature-G92
Terry Schiavo-I17
Testimony-E12
Testing-B17, C10
Tetraparesis-G95
Texas-G98
Text-Based Signatures-J12
THC and Metabolite-K2
THC-K55
The Alteration of Individual
    Handwriting-J8
The Council of Forensic Medicine-D27
Thermal Detector-A10
Thermal Imaging-J9
Thin Layer Chromatography-J18
Thinking Process-A6
Thiosulfate-G105
Third Molar-F1
Three-Dimensional Analysis-F7
Time-Stamp-B4
Time Since Death-H5, H8
Timeline Generation-B13
Time-Since-Death-H100
Tissue and Organ Harvesting-E14

Tissue Spatter-A11
Tool Marks-A11, A76, BS7
Tool Testing-B5, B8
Tool Validation-B5
Tooth-G109
Torture-H109
Touch DNA-A58, A62, E8
Toxic Metal Analysis-K12
Toxicology-D18, G16, G62, G119, K7,
    K13, K40, K47, K58, W5
Toxins-W9
Trace Analysis-A117
Trace Elemental Analysis-A73
Trace Elements-H11
Trace Evidence-A68, A107, A157, A182,
    A186, W4
Traffic Accidents-G85
Training-H65
Training and Exercising-H13
Trajectory-H110
Transfer-K57
Transmission-E17
Transrectal Absorption-G79
Trash Collector-D30
Trauma-G21, G42, H109
Trauma Analysis-H58
Trauma Timing-H116
Traumatic-G37
Traumatic Brain Injury-L2
Traumatic Injury-G110
Trends-D35, I5
Triage-H86
Trial Preparation-E4, L1
Trials-L1
Trigger Actuation Energy-C16
Trigger Pull-C16
Triggering Mechanism-A61
TriggerScan™-F6
Tsar Nicholas-LW1
Tsunami-F33
Turkey-D27, I4, I19
Twins' Handwriting-J5

## U

Ultrasonic-D11
Unidentified-LW7
Unidentified Decedent Database-S1
Unidentified Human Remains-D12
Unidentified Remains-F36
Unintentional Injuries-D3
Uniqueness-F13
United Nations-D28, D29
Unknown Microbes-G13
Unusual Methods-G103
Urine-K10, K54

Uterine Rupture-K27
UV LED Lights-F27
UV Microspectrophotometry-A75

## V

Vaccinations-W9
Vaginal Swabs-A109
Validation Technique-H61
Validation Testing For Daubert-J4
Validation-A45, A50, A92, A138, A139,
    G90
Vapor-A112
Vault-BS5
Vehicle Speed-C6
Vehicular Homicide-D18
Vertebrae-H27, H104
Veterinary Forensics-G34
Veterinary Science-G34
Victim Identification-F31, LW10
VICTIMS-D12
Video Footage-B22
Video Quality Assessment-B18
Virtual Digital Forensics-B23
Virtual Drug Analysis Drug-A183
Virtual Hair Comparison-A183
Virtual Lab-B23
Virtual Laboratory-A183
Virtual System-B5
Visual Examination-J6
Visualization-G76
Voice Stress-D36
Volatile Collection-A49
Volatile Organic Compounds-A37

## W

Western Australia-G73
Whole Genome Amplification-A87
Wild Game-G89
WinID-F31
Wood-A186
Worker Fatalities-G105
Work-Place Investigation-G2
Wound-G17
Writing Inks-J16, J18
Wrongful Conviction-D22, G97
WTC Dust-A179

## X

X Chromosome-A21
X-Ray Image Quality-F25
Xylazine-K44, K45

## Y

Y-Chromosome-A53, A89, A131
Y-Dropout-A89
Y-STR-A26
Y-Chromosomal STRs-A167
Year-of-Death-G101, H4
Yfiler-G26
YFSF-S3
YouTube-E13
Y-STR-A132, G26

## Z

Zolpidem-K19

# NOTES

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